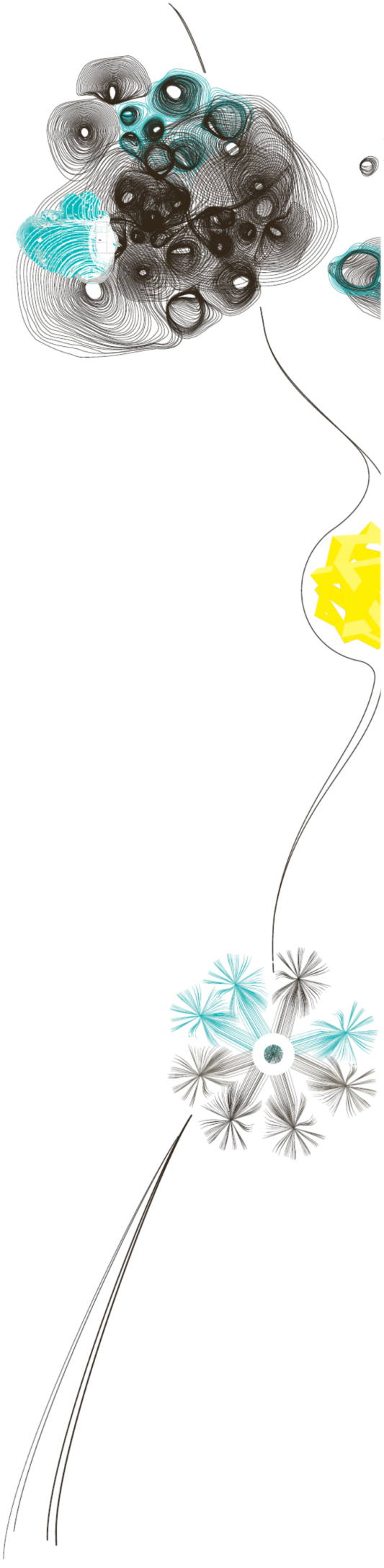

EXPLORING THE INFLUENCE OF VIGILANCE STATE ON MEP AMPLITUDE INDUCED BY TMS IN HEALTHY PARTICIPANTS AND STROKE PATIENTS

Medical Sensing and Stimulation

Master Thesis

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List of abbreviations

3D	3-dimensional
aMT	active motor threshold
APB	abductor pollicis brevis
CMAP	compound muscle action potential
CMCT	central motor conduction time
CT	computed tomography
CoV	coefficient of variance
CVA	cerebrovascular accident
ECG	electrocardiography/electrocardiogram
EEG	electro-encephalography/electro-encephalogram
EMG	electromyography/electromyogram
EPSP	excitatory postsynaptic potentials
FDI	first dorsal interosseous
FFT	Fourier transformation
HEOG	horizontal electro-oculogram
ICA	independent component analysis
IIR	infinite impulse response
MEP	motor evoked potential
MI	motor imagery
MRI	magnetic resonance imaging
PMCT	peripheral motor conduction time
rMT	resting motor threshold
SSEP	somatosensory evoked potential
TMS	transcranial magnetic stimulation
UMCG	University Medical Center Groningen

List of symbols

$\sim$	approximately
$\nabla\times$	curl operator
$\oint$	integration over a closed surface
α	significance level
μ	micro
σ	electric conductivity
τ	membrane time constant
Φ_B	magnetic flux
ϕ or V	electric potential
$\bar{A}_{doublepulse}$	mean amplitude obtained during double pulse stimulation
$\bar{A}_{triplepulse}$	mean amplitude obtained during triple pulse stimulation
$\overrightarrow{B}$	magnetic field
$d\phi$	electric potential derivative
$d\,\overrightarrow{l}$	infinitesimal displacement vector
$d\,\overrightarrow{S}$	small surface element perpendicular to
$\frac{d}{dt}$ or $\frac{\partial}{\partial t}$	(partial) time derivative
dx	spatial derivative
$\overrightarrow{E}$	electric field
$\overrightarrow{F}$	force
$\overrightarrow{J}$	electric current
p	critical alpha probability (p) factor
q	charge
R^2	coefficient of determination
$\overrightarrow{S}$	surface perpendicular to
S	surface
$\overrightarrow{v}$	velocity
$V(t)$	electric potential as a function of time
x	distance

Samenvatting

Doel Het voornaamste doel van deze studie was het onderzoeken van de invloed van vigilantie op de amplitude van de motor evoked potential (MEP) geïnduceerd door transcraniële magnetische stimulatie (TMS). Secundaire doelen waren om een MEP meetparadigma te ontwikkelen resulterend in minimale intra-individuele MEP amplitude variabiliteit en om vervolgens de uitvoerbaarheid van dit meetparadigma te onderzoeken in stroke patiënten.

Methode Deze studie was opgebouwd uit drie, elkaar opvolgende delen: een pilot studie, een studie in gezonde proefpersonen en een studie in stroke patiënten. In 20 gezonde proefpersonen werden MEP metingen geïnduceerd door TMS en elektro-encefalogram (EEG) registraties uitgevoerd. De stimulatie werd uitgevoerd met een intensiteit van 120% van de motorische drempelwaarde in rust, boven de vertex van de schedel. De MEPs geïnduceerd door deze stimulatie werden afgelezen van de eerste dorsale interosseus spier. Voor het minimaliseren van MEP variabiliteit veroorzaakt door veranderingen in spoelpositionering werd neuronavigatie apparatuur tijdens elk van de metingen gebruikt. Tijdens metingen in gezonde proefpersonen werden verschillende stimulatiemethoden gebruikt terwijl deelnemers verschillende taken uitvoerden om de invloed van vigilantie op de MEP amplitude te kunnen meten. Dit resulteerde in vijf verschillende condities: 1) single puls stimulatie tijdens ontspanning met ogen open, 2) single pulse stimulatie tijdens contralaterale precontractie, 3) single pulse stimulatie terwijl deelnemers een sprookje lazen waarover later vragen werden gesteld, 4) dubbel puls stimulatie en 5) driedubbel puls stimulatie. Om het vigilantie niveau te bepalen werd het frequentiespectrum van het EEG gedurende de 1400 ms voorafgaand aan iedere puls geanalyseerd. Om de invloed van het vigilantieniveau op de MEP amplitude te bepalen werden de resultaten van de frequentie analyse gecorreleerd aan de simultaan verkregen MEP amplitudes (Pearson's correlatie). Om te bepalen welke van de verschillende paradigma's resulteert in de minste variatie in MEP amplitude zijn coefficients of variance berekend, vervolgens zijn one-way ANOVAs gevolgd door Bonferroni gecorrigeerde testen uitgevoerd. Bij de twee geïncludeerde patiënten werden alleen MEP metingen gedaan om de uitvoerbaarheid van de meting te testen.

Resultaten Het paradigma wat significant een vermindering van variabiliteit in MEP amplitude teweeg bracht ($p = 0.005$) was de driedubbel puls stimulatie in vergelijking met het single pulse paradigma. Tevens was het opwekken van MEPs makkelijker door gebruik te maken van het driedubbel puls paradigma. Er was geen significante correlatie tussen de aanwezigheid van een bepaald hersenritme en MEP amplitude. Het driedubbel pulse stimulatie paradigma was goed uitvoerbaar in de twee geïncludeerde stroke patienten, echter niet bij beide patiënten werden MEPs verkregen.

Discussie De afwezigheid van een significante correlatie tussen vigilantie in de periode voorafgaand aan de stimulatie puls en de door dezelfde puls opgewekte MEP amplitude waarschijnlijk veroorzaakt door überhaupt weinig variatie in vigilantie. Een andere mogelijke verklaring hiervoor zou kunnen zijn de dat de vigilantie analyse methode achteraf niet bruikbaar bleek voor korte EEG fragmenten. Daarnaast zou de afwezigheid van correlatie veroorzaakt kunnen zijn door verstoringen in de delta en theta frequentiebanden door beweging van hoofd en ledematen veroorzaakt door de stimulatie. Voor vervolgonderzoek wordt daarom geadviseerd om het hoofd van de patiënt te fixeren in een bepaalde positie om bewegingsartefacten ten gevolge van de stimulatie zoveel mogelijk te voorkomen.

Conclusie Variabiliteit in MEP amplitude wordt verminderd door gebruik te maken van driedubbel puls stimulatie. Tevens is het opwekken van MEPs makkelijker door gebruik te maken van het driedubbele puls paradigma in vergelijking met het single puls paradigma. Een invloed van vigilantie op MEP amplitude kon niet worden bevestigd in deze studie, echter dit kan veroorzaakt zijn door onbekendheid met TMS van de proefpersonen waardoor ze erg schrikkerig reageerden op de stimulatie met als gevolg grote verstoringen in het EEG. Verder was de uitvoerbaarheid van het driedubbel puls paradigma goed in de geïncludeerde stroke patienten. Echter, een definitieve conclusie over de uitvoerbaarheid op basis van slechts twee patienten kan aan de hand van deze studie niet getrokken worden.

Abstract

Objective The main objective was to investigate the influence of vigilance state on motor evoked potential (MEP) amplitude induced by transcranial magnetic stimulation (TMS). Secondary objectives were to develop a stimulation paradigm to minimize intraindividual MEP amplitude variability, and to subsequently test the feasibility of this newly developed paradigm in stroke patients.

Methods This study was composed of three consecutive parts: a pilot study, a main study in healthy participants, and a feasibility study in stroke patients. A total of 20 healthy participants were included in this study and were subdued to MEP measurements induced by TMS with simultaneous electroencephalogram (EEG) recording. Stimulation was done at 120% of resting motor threshold intensity over the vertex of the scalp inducing MEPs that were derived from the first dorsal interosseous muscle. In order to minimize MEP variability due to differences in coil positioning, neuronavigation was used throughout all measurements. During each measurement participants were instructed to perform different tasks and were subdued to different stimulation methods to investigate the influence of vigilance state on MEP amplitude. This resulted in five different paradigms: 1) single pulse stimulation while relaxing with eyes open, 2) single pulse stimulation during contralateral precontraction, 3) single pulse stimulation while reading a fairy tale (this task was followed by a short tale-based questionnaire), 4) double pulse stimulation, and 5) triple pulse stimulation. For exploring vigilance state in healthy participants, the frequency spectrum of the EEG in periods of 1400 ms preceding each magnetic pulse was analysed. To investigate the influence of vigilance state on MEP amplitude, results obtained from vigilance state analysis were correlated to simultaneously acquired MEP amplitudes using Pearson's correlation tests. For determining which paradigm resulted in the lowest MEP amplitude variability coefficients of variance were calculated, subsequently one-way ANOVAs followed by Bonferroni corrected tests were performed. In the two included stroke patients, only MEP measurements induced by TMS were performed to test the feasibility of the optimal paradigm for minimizing MEP amplitude variability.

Results A significant decrease in MEP amplitude variability ($p = 0.005$) was obtained through stimulation using the triple pulse paradigm in comparison to the single pulse paradigm. In addition, MEPs were more easily obtained using triple pulse stimulation in comparison to single pulse stimulation. Furthermore, a significant correlation between the presence of a specific brain rhythm and MEP amplitude could not be found. Moreover, the patient study demonstrated the triple pulse paradigm to be feasible for use in the two included stroke patients. However, MEPs could not be obtained during all measurements.

Discussion The absence of significant correlations between EEG based pre-MEP vigilance state and MEP amplitude is probably caused by low variability in vigilance state in the first place. Another possible explanation could be that the vigilance state analysis method appeared to be unusable retrospectively for application in short EEG fragments. In addition, the lack of correlation could be due to interference of head and limb movements in the delta and theta bands caused by the stimulation. For further research it is therefore advised to keep the participants' head still through fixation in a certain position to minimize movement artefacts caused by the stimulation.

Conclusion MEP amplitude variability is decreased in healthy participants when using the triple pulse stimulation paradigm. In addition, MEPs are more easily obtained using triple pulse stimulation compared to single pulse stimulation. An influence of vigilance state on MEP amplitude could not be confirmed, however this could be caused by unfamiliarity with magnetic stimulation and tenseness during TMS-MEP measurements. This led to undesired limb movements influencing EEG recordings overshadowing a possible correlation between vigilance state and MEP amplitude. Furthermore, using the triple pulse paradigm in the included stroke patients was feasible. However, MEPs could not be obtained in all measurements. A solid conclusion regarding the feasibility of the triple pulse paradigm in stroke patients cannot be drawn based on the inclusion of merely two patients.

1 Introduction

Worldwide, the second most frequent cause of death is stroke. In addition, it is a major cause of long-term adult disability in Western countries [14,37,62]. Prevention, diagnosis and treatment of stroke have significantly improved over the last decades, but advances in rehabilitation after stroke have remained modest [6,62]. Recovery of motor function, particularly in the upper limb, is essential for independence in activities of daily living [9,72,73].

Nowadays, in addition to medical management after acute stroke to prevent further cerebral damage, stroke rehabilitation is initiated early with the ultimate goal of achieving better recovery in the first months after stroke and reducing disability during the years that follow [72].

For early initiation of rehabilitation an accurate prognosis and an individualized program should be made. However, setting up an individualized program is in current practice rather difficult. The goals, type, and duration of rehabilitation after stroke in the middle cerebral artery territory, depend highly on the estimated recovery time of motor function for each patient. Consequences of the decisions made regarding patient recovery are extensive, but are nowadays made without objective information concerning the patient's capacity for motor recovery [62]. In order to realise early initiation of effective rehabilitation the individual's potential for recovery should be established first. This would subsequently enable setting realistic goals and more efficient allocation of resources [6,62].

Despite many advances in improving prevention, diagnosis and treatment of stroke, dependency rate after stroke still reaches 20-30%. Currently, functional motor impairment after stroke is determined on the basis of clinical assessment, such as the Canadian Neurological Scale for assessing and monitoring the neurological status of stroke patients [8], the Medical Research Council scale for testing muscle strength [23], the Fugl-Meyer Motor Assessment test [20,29], the Barthel Index Scale and the Modified Rankin Scale for measuring disability [39,63,72], and the Frenchay Arm Test for upper extremity proximal motor control [29,73].
The worst prognosis for motor outcome is seen in patients with initial paralysis, however, some of these patients will show partial or even complete motor recovery. Since postlesional recovery appears strongly influenced by residual spared function, it is important to objectively quantify the severity of functional motor impairment [29]. Clinical examination alone lacks the ability to detect the potential for motor recovery as patients cannot voluntarily perform or poorly perform any of the test-related tasks (especially in noncooperative or severely cognitively impaired patients) [29].

A method that could be used to predict motor recovery after stroke is transcranial magnetic stimulation (TMS). TMS is capable of revealing changes in cortical excitability after stroke [41]. Recent studies have investigated the presence of motor evoked potentials (MEPs) in the paretic limb in response to stimulation of the affected hemisphere [41,52,62]. The presence of such MEPs in the first week after stroke, was found to be predictive of good motor recovery. Contrarily, absence of MEPs during initial examination, predicted poor recovery [41]. Other studies investigated the prognostic value of the change in MEP amplitude over time in the acute phase post stroke [3,47]. However, the heterogeneous methodologies applied in the latter studies complicate the interpretation and comparability of their results. Furthermore, interindividual and intraindividual MEP variability may have hampered the interpretation of MEP changes over time.

In literature, several sources of MEP variability have been identified and studied. Sources of variability in

MEP amplitude are for example fluctuations in motor-related cortico-cortical connectivity strength [15], and influence of coil positioning [42]. Stimulus intensity [42] and precontraction of the ipsilateral and contralateral response have been described to be of influence on MEP amplitude, as well [5, 21]. Furthermore, a study by Anderson et al. (1999) investigated motor pre-excitation through observation or through motor imagery (MI) and stated these phenomena also to be a source of MEP amplitude variability. In addition, certain types of medication as well as nicotine might influence MEP amplitude by affecting neuronal excitability [24, 48, 65, 75, 77].

There is another possible source of MEP amplitude variability that has not yet been studied extensively. A previous study at the department of clinical neurophysiology at the University Medical Center Groningen (UMCG) executed by Susan van Maarseveen (2009) suggested that a person's cortical activation state may have significant impact on MEP amplitude.

Different activation states of cerebral cortex have been described to influence cortical excitability. For instance, an increase in beta activity due to a higher level of attention [49] has been said to result in increased cortical motor excitability [35,57]. To describe the various activation states different terms have been used, for example arousal, alertness, vigilance, and attention. The most common usage is sustained attention or tonic alertness. Scientifically the term vigilance state has been used to refer to arousal level on the sleep-wake axis [49].

A study investigating the relation between sustained attention and electro-encephalogram (EEG) measurement suggested a relationship between the two [49]. Here, they described that worse sustained attention is often correlated with a lower level of attentiveness corresponding to increased theta and decreased beta activity (with the decreased beta activity the most useful parameter) [49].
Another study investigated the modulatory effect of vigilance states on cortical excitability [43] and found a strong modulatory effect of different states on the sleep-wake axis (here wake, sleep deprivation, and sleep) on the presence of different brain rhythms [43].
Furthermore, effects of brain synchronization on MEP amplitude have been explored by Ferreri et al. (2014). They also describe the influence of vigilance state on brain rhythms.

These studies in addition to the results of the earlier study at the UMCG have raised the question how vigilance state modulates MEP amplitude. To our knowledge this has not yet been investigated correcting for all other factors influencing MEP variability.

Summarizing, it has been shown that the presence of MEPs is indicative for recovery after stroke. However, although it may be expected that the change in MEP amplitude over time in the acute phase post stroke can provide additional information about the extent of recovery, results so far have been inconclusive [3, 47]. Therefore, this study aimed to minimize intraindividual MEP amplitude variability by developing a new stimulation paradigm which would, in the long term, enable evaluation of the change in MEP amplitude in the acute phase post stroke.

1.1 Primary and secondary objectives

We propose that, due to inherent variability in intraindividual MEP amplitude, interpretation of MEP amplitude variability over time in stroke patients has been hampered. Since vigilance state may be a source of MEP amplitude variability that is still poorly understood, we here investigate the relation between the level of alertness and MEP amplitude. The primary aim was to investigate the influence of vigilance state on MEP amplitude in healthy adults. Secondary objectives were to develop a paradigm

to stabilize the influence of vigilance state resulting in lower MEP amplitude variability in healthy adults and to test the feasibility of this paradigm in stroke patients.

2 Stroke: pathophysiology and recovery

2.1 Stroke

In developed countries worldwide, stroke is the second most common cause of death and the third most common cause of long-term adult disability [14, 37, 62].
(According to Kumar and Clark's book of clinical medicine) Stroke can be defined as "a syndrome of rapid onset of cerebral deficit (usually focal) lasting >24 hours or leading to death, with no cause apparent other than a vascular one" [37].

Risk factors that have consistently been identified as important predictors of stroke outcome are: age, hypertension, antihypertensive treatment, alcohol intake, previous stroke, and whether the patient is diagnosed with atrial fibrillation. Other associated risk factors that increase the likelihood of getting a cerebrovascular accident (CVA) include: smoking, diabetes, coronary heart disease, left ventricular hypertrophy, excessive alcohol intake, and family history of stroke [60].

2.2 Pathophysiology

The two major types of stroke are cerebral ischaemia and haemorrhage. In general, infarcts caused by local atheromatous vascular changes (leading to local thrombosis and occlusion) are ischaemic. When peripheral atheromatous vascular changes lead to thrombosis and subsequent embolization, stroke is referred to as heamorrhagic. High prevalence and progressive nature of this precursor is reflected in the high morbidity and mortality that is associated with cerebrovascular occlusive disease [56].

The difference between haemorrhagic infarction and ischaemic infarction is that haemorrhagic events are caused by embolization, whereas ischaemic infarction occurs as a result from atheromatous vascular changes. Embolization leads to abrupt occlusion of vascular flow, subsequent necrosis of distal affected blood vessels, resulting in blood leakage in the region. In contrast, thrombosis progresses more slowly and causes a gradual deprivation of blood flow in the downstream arteries [56].

In order to fully understand the main stroke syndromes and why occlusion of different cerebral vessels causes diverse neurologic deficits, knowledge of common arterial anatomy and likely sites of atheromatous plaques and stenosis is helpful. Figure 1 displays the arteries supplying the brain and spinal cord.
Occlusion of different cerebral vessels causes diverse neurologic deficits, depending on the exact location of the occlusion. For example, lengthy and slender striate arteries originating from the proximal middle cerebral artery (see the upper right side of figure 1) are commonly involved in ischaemic infarction; often resulting in hemiparesis or hemiplegia. Whereas the trifurcation of the middle cerebral artery (see the upper left side of figure 1 where the middle cerebral artery divides in the posterior communicating artery, the anterior communicating artery and the internal carotid artery) is a common site for lodgement of emboli [56]. Often when the dominant hemisphere is involved in the infarction, aside from motor and sensory deficits, aphasia is a common clinical manifestation.

2.3 Post stroke

In the acute phase post stroke, when thrombolytic therapy is necessary, emergency treatment is key. This treatment has been shown to improve outcome and should be used immediately to improve tissue re-perfusion. However, the benefit of thrombolysis decreases with time, even within a time window of 4.5 hours [37].
In the acute phase post stroke, rescue mechanisms of the brain will occur. Capillaries will proliferate and will attempt to increase perfusion of the infarct penumbra. The penumbra represents a swollen

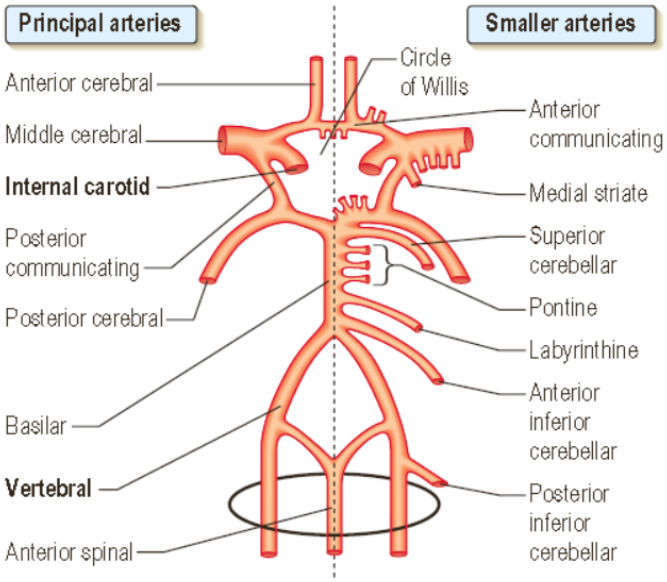

Figure 1: illustration of the 'Circle of Willis' and the supplying arteries [37]

area of brain tissue surrounding the infarct which is at risk of progressing to infarction also, but is still salvageable if re-perfused. The penumbra does not function, but is structurally intact and can be detected with magnetic resonance imaging (MRI) [37, 56]. Ultimately, within the area of infarction, hypoxia leads to neuronal damage. A drop in adenosine triphosphate is accompanied by the release of glutamate, which opens calcium channels causing the release of free radicals. These alterations in tissue metabolism will lead to inflammatory damage, necrosis and apoptotic cell death. The necrotic fragile debris of the area of infarction will be phagocytosed and eliminated by macrophages [37, 56].
Investigations to confirm diagnosis are non-contrast computed tomography (CT) which will demonstrate haemorrhage immediately. Cerebral ischaemic infarction, however, is often not detected immediately or only subtle changes are seen in the acute phase post stroke [37]. MRI does show early changes due to ischaemic infarction, nevertheless, a CT scan is acquired first since this imaging technique is more readily available and consequently a delay in giving thrombolysis if necessary can be prevented [37].

2.3.1 Motor impairment and rehabilitation

One of the most frequent defects seen post stroke is upper-limb motor impairment; a deficit with a significant impact on the quality of life and independence in daily activities [9, 37, 72, 73]. Particularly during working life, it alters the patient's remaining years radically [37].
After ischaemic stroke and accompanying decrease in motor function, a variable degree of motor recovery is seen [64]. Occurrence of variability in the nature and extent of recovery of upper limb functionality and motor function is well recognized [38], and as a consequence, individual patients need different rehabilitation goals and schedules [9, 62].

Currently, despite of stroke being one of the leading causes of death, development of patient-based recovery and rehabilitation methods in the acute phase post stroke is not satisfactory [6, 62].
Early rehabilitation in the acute phase post stroke is clinically advised, since physiotherapy has particular value in the first few weeks [37]. However, prognostic models have not gained much acceptance due to doubts regarding their predictive accuracy. Observation bias, and problems with generalization of results are examples of issues that impede the efficacy of using predictive models [72, 73].

In order to provide optimal individualized recovery and rehabilitation methods in the acute phase post stroke, upper limb recovery should be predicted using a set of well-defined objective variables to reliably establish functional motor impairment. These data would provide initial conditions on which rehabilitation physicians and physiotherapists can base individualized programs and methods for rehabilitation to allow optimal regain of motor function [6, 9].

The grade of paresis is one variable on which motor recovery in the early stages post stroke could be predicted [9]. An existing technique, TMS, can be used to obtain information on the conductivity of motor pathways of the central nervous system. This technique, an electrophysiological tool, is used to stimulate the brain cortex (particularly the primary motor area) to obtain information about the functionality of the motor pathways. This information can be acquired in the form of MEPs derived from the muscles of interest [9]. This measurable variable may be suitable to establish functional motor impairment and may be helpful in the development of patient-based rehabilitation methods [9].
TMS and the MEPs that can be induced by TMS will be further explained in the next two chapters.

3 Transcranial Magnetic Stimulation

TMS is a technique used for stimulation of nervous tissue by inducing an electric field by electromagnetic induction. One of the reasons this technique is favoured over direct electrical stimulation is that it is less painful [70].
For understanding the underlying mechanisms of TMS a brief introduction on electromagnetism is given. For a more elaborate explanation, please refer to Appendix A.

3.1 Basic theory of electromagnetism

In 1831, Faraday attempted to use static magnetic fields to produce a steady electric current in a loop of wire. He did not succeed, but observed that when a magnet was brought close to the coil or removed from the coil currents did flow momentarily [25]. Here, the magnetic flux near the coil changes with time, inducing an electric field in the coil. This electric field is proportional to the rate of change of the magnetic field, which results in free charges flowing in the coil [19, 25]. This process is described by:

$$\oint \overrightarrow{E} \cdot d\overrightarrow{l} = -\frac{d\Phi_B}{dt}. \tag{1}$$

Here, $\overrightarrow{E}$ is the electric field that does the work of moving the electrons in the wire, $d\overrightarrow{l}$ is the element of displacement along the path of integration, and $-\frac{d\Phi_B}{dt}$ represents the change in magnetic flux with time. This equation relates the changing magnetic flux to the electric field it produces. The integral sign on the left indicates the boundary surrounding the path enclosing the area through which the magnetic flux is changing. The minus sign reflects that the currents flow in opposite direction from the change in magnetic flux.

When the flux is written in terms of the magnetic flux density, the following equation is obtained [22, 25]:

$$V = \oint_S \overrightarrow{E} \cdot d\overrightarrow{l} = -\frac{d}{dt}\int_S \overrightarrow{B} \cdot d\overrightarrow{S}. \tag{2}$$

Here, the integration is over a surface S defined by the coil and V represents the electric potential. Without movement of the coil, S does not change over time, and the equation can be rewritten to:

$$V = \oint \overrightarrow{E} \cdot d\overrightarrow{l} = -\int_S \frac{\partial \overrightarrow{B}}{\partial t} \cdot d\overrightarrow{S}. \tag{3}$$

Here, $\frac{\partial \overrightarrow{B}}{\partial t}$ represents the change in magnetic field with time. Equation 3 is also known as Faraday's law of electromagnetic induction in integral form. This form may be regarded as misleading since it describes two distinct phenomena: magnetic induction (due to a changing magnetic field) and electromotive force (due to movement of a charged particle through a magnetic field) [17]. Both phenomena cause the arising of an electromotive force, however, solely magnetic induction produces an electric field. Another version of Faraday's law, the differential version, separates the two effects [17, 25]:

$$\nabla \times \overrightarrow{E} = -\frac{\partial \overrightarrow{B}}{\partial t} \cdot d\overrightarrow{S}. \tag{4}$$

Here, $\nabla\times$ represents the differential curl operator.

3.2 Electromagnetism and TMS

In case of TMS, electrical charge is stored in capacitors. This stored energy is periodically discharged from the capacitors through a conducting coil and produces a time-varying electrical field. In turn, this electrical field produces a transient magnetic field, causing a current to flow in a nearby conducting material, such as neurons. When of sufficient strength, this current may produce depolarisation of the conducting brain tissue located just under the coil [16]. The latter is illustrated in the following figure:

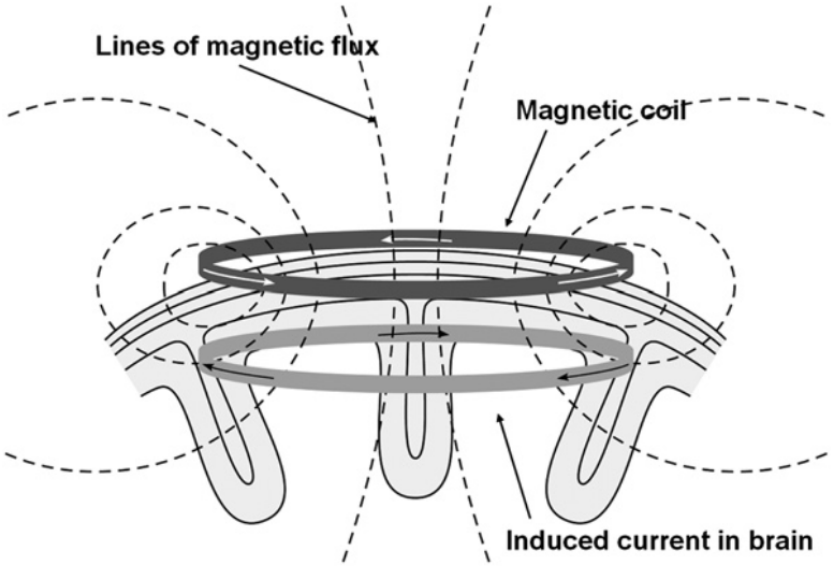

Figure 2: illustration of the current in the brain induced by TMS [24]

In magnetic stimulation, a brief, high intensity pulse is produced in a coil of wire. The magnetic field is produced perpendicular to the plane of the coil (placed tangential to the scalp). Perpendicular to the produced magnetic field, an electric field is induced. The voltage of the field itself may excite neurons, however likely more important for neuronal excitation are the induced current flows by the electric field [24].

Thus, an electrical field applied to neurons causes accumulation of induced current flows by the nerve membrane which may lead to excitation of these cells, resulting in driving of an intracranial electrical current in the brain by the electric field [17, 25, 33, 50]:

$$\overrightarrow{J} = \sigma \overrightarrow{E}. \tag{5}$$

Here, $\overrightarrow{J}$ is the current, and σ is the electrical conductivity of the brain [25].

3.3 Physiology

The flowing currents induced by TMS (see figure 2), may enter and exit the neurons. When currents remove charges from the membrane, a decrease in membrane potential will occur followed by depolarisation. The charge on the membrane capacitance increases when currents enter the cell membrane, leading to hyperpolarisation [70].

In case of sufficient membrane depolarization, an action potential may be initiated. Whether an action potential is initiated depends on the spatial shape of the cell, the characteristics of the electric field, the coil geometry, and its position and orientation with respect to the nerve fibers [55, 70]. The magnitude and time-course of the gradient of the component (of the induced electric field) parallel to the nerve fibers has been shown to determine whether and where stimulation occurs [55]. The membrane current responsible for depolarisation or hyperpolarisation, is maximum where the spatial gradients of the axial current and the induced electric field are the largest. When applying a uniform field along an ideal

cylindrical cell, membrane depolarisation or hyperpolarisation will not occur; no spatial gradient exists which is required for stimulation [55]. In reality however, uniform applied fields often stimulate neurons anyway. This is possible due to bends in the cell membrane: here, axial currents are non-zero and induction of membrane currents occurs, which may produce excitation or inhibition if sufficiently large [70].

The underlying mechanism of TMS is induction of an electric field in brain tissue for the purpose of neuronal excitation. In order to induce sufficiently strong motor cortex activation to lead to measurable MEPs, the cortical electric field should be in the order of 100 V/m. Cortical conductivity is $\sigma = 0.4$ m/s, thus an electric field strength on the order of 100 V/m results in a cortical current density of approximately 40 $\mu A/mm^2$ [25] (calculated through use of equation 5).

4 Motor evoked potentials

The MEPs induced by TMS, can be used to study the physiology of corticospinal conduction in healthy subjects as well as in patients with central nervous system pathologies. Parameters of MEPs that can be studied include the latency yielding the central motor conduction time (CMCT), the amplitude, area under the curve of the MEP amplitude, and the stimulation threshold, silent period, and facilitation [75]. This study aimed to investigate the influence of vigilance state on MEP amplitude in healthy adults, and to subsequently develop a paradigm to stabilize the influence of vigilance state resulting in lower MEP amplitude variability to subsequently be tested for feasibility in stroke patients.
In order to focus on minimizing MEP amplitude variability during measurement through minimizing influence of vigilance state, all other influences should be controlled for as much as possible.

4.1 Factors influencing MEP amplitude

When stimulating a peripheral nerve, the resulting muscle response is termed the compound muscle action potential (CMAP). The CMAP describes the number of activated motor units, which approximately corresponds to the number of activated motor axons [75].
In theory, the MEP amplitude induced by TMS is also related to the number of activated corticospinal motor neurons. However, this correlation is unclear due to different physiological mechanisms [75]. Some of these physiological factors and other factors influencing MEP amplitude are described in the following paragraphs.

4.1.1 Motor factors influencing MEP amplitude

The influence of active or relaxed muscles
The descending corticospinal currents evoked by TMS result in excitatory postsynaptic potentials (EPSPs) at the motor neuron. Especially at high intensity stimulations, motor neurons are discharged asynchronously which leads to a MEP of longer duration. At slightly different latencies, cancellation between phases of motor units can occur [75]. In active and relaxed muscles, latencies of MEPs can differ; this results from the time it takes for the released EPSPs induced by TMS at the motor neuron to depolarize this motor neuron to its firing threshold.

Mainly due to increased excitability of spinal motor neurons during contraction, a difference between MEP amplitudes at rest and during activation occurs. Thus, a certain amount of descending excitation causes more spinal motor neurons to discharge in a contracted muscle state, leading to a larger MEP amplitude [75].

Facilitation through muscle precontraction may be useful clinically, as the stimulation threshold in contracted muscles is decreased. In patients with diseases affecting the central nervous system, reproducible MEPs in relaxed muscles can be difficult or even impossible to obtain. However, a major pitfall of this technique is that precontraction of the target muscle is only possible if the patient is capable of muscle contraction [5]; stroke patients, for example, are often not capable of contracting the target muscle due to paresis.

A possible solution for this problem was examined by Gorsler et al (2003). They investigated the effects of right motor cortex repetitive stimulation on the excitability of the contralateral left motor cortex. The main finding of this study was that following repetitive stimulation of the right hemisphere, the net cortical excitability of the left hemisphere was increased as reflected in an increase in MEP amplitude [21]. This is also called the intercallosal effect which indicates a connection between the left and

right hemisphere [4, 11, 53]. This implies that contralateral precontraction allows to induce a facilitatory effect in stroke patients similar to ipsilateral contraction in persons without diseases affecting the central nervous system.

The influence of double pulse TMS and triple pulse TMS
In 2007, Mark Hallett (2007) described that paired pulse stimulation [76], in which a subthreshold conditioning pulse and a second test stimulus above stimulus threshold are applied, results in cortical facilitation at interstimulus intervals between 8 and 30 ms [24].
Furthermore, Sacco et al. (2009) studied the difference between double pulse TMS responses and triple pulse TMS responses at different interstimulus intervals. Here, two or three Magstim 200 units were used connected to a single coil to perform the measurements. Interstimulus intervals varied between 1.5, 2.0 and 3.0 ms and stimulus intensities varied between below active motor threshold (aMT), a combination of below aMT and at aMT, as well as all at aMT. Triple pulse TMS was concluded to have a more powerful facilitatory effect on MEP compared to double pulse TMS [58].

The influence of MI
Watching movement of a certain body part in others or imagining to perform a movement of a certain body part may enhance MEP amplitude of the involved muscles in the observed or imagined action. This phenomenon is called motor facilitation or MI. MI is said to be the result of a mirror neuron system mediated mechanism, which results in an increase in excitability of the primary motor cortex [51, 66].

4.1.2 Chemical factors influencing MEP amplitude

The influence of drugs
There is evidence that neurotransmitters (glutamate, GABA) and modifiers of neurotransmission (dopamine, norepinephrine, serotonin or acetylcholine) influence MEP amplitude. MEP amplitude was found to decrease after application of allosteric modulators of the GABA A receptor (benzodiazepines, barbiturates), as well as after application of dopamine agonists (cabergoline), and after a norepinephrine antagonist (guanfacine) [77]. In contrast, after application of dopamine antagonists (haloperidol), as well as various norepinephrine agonists (methylphenidate, d-amphetamine, reboxetine, yohimbine), a serotonin re-uptake inhibitor (sertraline), or a muscarinic receptor (M1) antagonist (scopolamine) MEP amplitude increased [77].

The influence of nicotine
Lang et al. (2008) investigated cortical excitability in chronic smokers and stated that smokers display reduced intracortical facilitation accompanied by lower MEP amplitudes [40]. Since MEP amplitude is a global measure of motor-cortical excitability (modulated by ion channel activity, GABAergic, glutamatergic, dopaminergic, adrenergic, and cholinergic receptors [77]) the indirect influence of nicotine on various receptors makes it impossible to draw definite conclusions regarding the working mechanism of how nicotine reduces cortical excitability [40, 65].

4.1.3 Other factors influencing MEP amplitude

The influence of age
In a study by Eisen et al. (1991), MEP amplitude's age-dependency was investigated. Here, a linear decline as a function of age in maximum and mean amplitude was observed. This decline was thought to be caused by a linear decline as a function of age in the number of motor units in a muscle [12].

The influence of variable cortico-cortical connectivity strength

MEPs, when induced by a train of consecutive individual stimuli, show fluctuations in amplitude when recorded from a muscle at rest while the induced movement in the same muscle does not change [15]. Changes in motor cortex excitability are believed to be responsible for these MEP amplitude fluctuations. Ferreri et al. (2014) speculated that fluctuations in MEP amplitude can be correlated with spontaneous EEG oscillatory synchronization power; i.e., spontaneous cortical oscillations are correlated with rhythmic changes in neuronal membrane potentials, thus with changes in neuronal excitability.

The influence of mental effort

Amplitudes of MEPs recorded from forearm muscles in participants looking at a target and performing mental calculations, were higher as opposed to when participants had their eyes closed and were trying to avoid any mental activity [54].

Andersen et al. (1999) suggest arousal to have a (partial) facilitatory effect on MEPs as well.

Technical influences on MEP amplitude

Another source of MEP variability is coil positioning. The size and latency of the MEP can be significantly altered due to even a slight positional change or rotation of the stimulation coil [34, 36, 42, 75]. Furthermore, variance in stimulus intensity also causes variability in MEP size [36, 42].

5 Materials and methods

5.1 Study set-up

In order to investigate the influence of vigilance state on MEP amplitude, this study was composed of three consecutive parts. First a pilot study in five healthy participants was conducted, next a sample of 15 healthy participants was recruited. Thereafter a feasibility study in stroke patients was done.

For the pilot study, a sample of five healthy participants was recruited to test the feasibility of simultaneous operation of all different techniques used during a measurement. In addition, the obtained EEG signal containing TMS pulse artefacts was visually qualitatively assessed and experiences of the first participants were taken into account before proceeding to the main study in healthy participants.

Subsequently, in a larger group of 15 healthy participants EEG and electromyography (EMG) were recorded during five different MEP stimulation paradigms to study the influence of vigilance state on MEP amplitude and investigate MEP amplitude variability. Finally, the paradigm which resulted in the least MEP amplitude variability was selected for further study of feasibility in stroke patients.

5.2 Participants

Healthy participants had to meet the following inclusion criteria:

- Perceived healthy
- 18 years or older
- Informed consent

Exclusion criteria that were taken into account were:

- Taking medication influencing cortical excitability
- Presence of metals in the head
- Presence of electronic implants
- (Suspected) history of epilepsy
- History of severe cerebral trauma
- (Possible) pregnancy

Stroke patients had to meet the following inclusion criteria:

- 18 years or older
- Informed consent
- One sided paresis of the arm and/or leg as concluded by the attending physician
- First ever stroke
- Ischaemic stroke

Exclusion criteria that were taken into account in addition to the previous mentioned exclusion criteria for healthy participants were:

- Presence of cardiac lines
- Clinical manifestations of aphasia

All participants gave their informed consent for the study which was approved by the Ethics Committee of the UMCG.

5.3 Experimental paradigm

5.3.1 Healthy participants

During each session, participants were seated in an upright position on the treatment couch. An active EMG electrode was attached over the belly of the first dorsal interosseous (FDI) muscle of the non-dominant hand; this muscle was chosen as the largest MEPs can be captured from this muscle [51].

A reference EMG electrode was attached over the metacarpophalangeal joint with a ground electrode on the dorsolateral side of the hand (as illustrated in figure 3). MEPs were measured with a Synergy (Synergy NCS EMG EP IOM system, Optima Medical Ltd, Guildford, Surrey, UK) measurement system.

Figure 3: illustration of the electrode placement for MEP measurement from the FDI muscle [2]

The black electrode was placed on the belly of the FDI muscle; the red electrode was placed on the metacarpophalangeal joint; the green electrode is the ground electrode.

The non-dominant motor cortex (as evaluated by the Handedness Questionnaire [71]) was magnetically stimulated with a Magstim Rapid 2 (Magstim Company Ltd, Whitland Wales, U.K.), with a circular coil. The scalp area over the vertex was explored to find the part of the motor cortex representing the FDI muscle. The intensity of the magnetic stimulation was adjusted (starting with 50% of maximum stimulator intensity and increased by 5% at a time) to find the resting motor threshold (rMT). The rMT was defined as the minimum magnetic intensity necessary to elicit a visible MEP of at least 50 μV in 5 out of 10 consecutive stimulations.

The five incorporated different measurement tasks can be subdivided into two groups, namely behavioural adjustments to the standard MEP measurement paradigm and technical adjustments to the standard paradigm. The different paradigms included were:

- Standard MEP measurement: during this task participants were instructed to relax and keep their eyes open. MEPs were obtained using single pulse stimulation.

Behavioural adjustments:

- Contralateral precontraction: during this task the investigator instructed the participant into making a fist with the dominant hand just prior to single pulse stimulation and obtaining a MEP from the non-dominant hand.
- Reading a fairy tale: during this task participants were instructed to read a fairy tale. Simultaneously MEPs were obtained using single pulse stimulation. Following this task, participants were asked to answer a few questions to assess whether they properly read the fairy tale (i.e., whether attention was sustained throughout the measurement).

Technical adjustments:

- Double pulse stimulation: during this task participants were instructed to relax and keep their eyes open. MEPs were obtained using double pulse stimulation.
- Triple pulse stimulation: during this task participants were instructed to relax and keep their eyes open. MEPs were obtained using triple pulse stimulation.

TMS was applied at 120% of rMT throughout all tasks. Per task, 20 visible MEPs of at least 50 μV were obtained; this was assessed visually during measurements. All measurements were conducted with similar stimulation intensity and interstimulus intervals were kept constant during double and triple pulse measurements (preferably at the optimal stimulus interval of 35 Hz corresponding to 28.6 ms [24], otherwise the highest frequency the stimulator would allow was chosen).

An MRI compatible EEG cap with 29 Ag/AgCl EEG electrodes was placed on the participant's head and filled with conductive paste. The EEG was recorded using BrainVision Recorder software (Version 2.0, Brainproducts, Munich, Germany). The EEG was amplified using an MRI compatible amplifier (BrainAmp MR plus, Brain Products GmBH) so that TMS artefacts (see figure 5) would not saturate the EEG signal.

To minimize MEP variability due to variation in coil positioning, neuronavigation was used during all measurements using SofTaxic Optic 2.0 software – neuronavigation system for TMS (E.M.S. srl, Bologna, Italy) with an optical navigation system (Polaris, Spectra, Northern Digital Inc.) consisting of a camera and movement sensors consisting of reflective spherical markers. Using neuronavigation, the optimal coil position ('hotspot') was stored. This system enables real-time visualization of the coil location in relation to certain predefined landmarks on a computer screen. To make it work a 3-dimensional (3D) reference tool containing spherical markers was attached to the handle of the TMS coil. Orientations of the coil with respect to the reference tool were defined with a 3D pointer (stylus). The position sensor of the camera emits infrared light which is reflected by the passive spherical markers of which an additional four are mounted on the participant's head using an elastic headband. Landmarks were defined by the investigator with the 3D stylus and subsequently stored. The landmarks were the nasion, and the left and right pre-auricular point. Coil position was retained throughout the session by visual assessment.

The measurement set-up is displayed in figure 4.

Figure 4: illustration of the measurement set-up

On the left, the neuronavigation laptop (1) is shown. The accompanying camera (2) can be observed on the right, and the accessories containing spherical markers (3) are shown near the center of this figure. On the left and right, two computer screens (4) forming part of the Synergy EMG measurement system can be observed. The accompanying electrode output panel (5) is shown near the center of this figure. With the laptop positioned on the right (6) accompanied by the MR compatible amplifier set-up (7) and the EEG cap (8), EEG was recorded. On the left, the conductive paste and other accessories (9) used for the preparation of EEG recording is shown. The Magstim stimulator (10) is shown on the right of the figure as well as its accompanying round coil (11).

5.3.2 Stroke patients

In stroke patients, measurements were conducted in the same manner as for healthy participants with a few adaptations. Stroke patients were seated in an upright position in a wheelchair and were instructed to relax and keep their eyes open. EEG was not measured, solely MEP measurements induced by TMS were conducted. To determine rMT, single TMS pulses were administered. After storage of the hotspot using neuronavigation, only the triple pulse stimulation paradigm was tested to investigate its feasibility in these patients. The choice for the triple pulse stimulation paradigm was made on the basis of the results described in section 6.1.2.

5.4 Data analysis

An overview of the analysis procedure of EEG and MEPs is described in the following paragraphs. For a more elaborate explanation including illustrations of the EEG analysis steps taken in BrainVision Analyzer as well as the MEP analysis steps in Synergy, please refer to Appendices C and D.

5.4.1 EEG analysis

The frequency spectrum of the EEG during 1400 ms preceding each magnetic pulse was analysed in BrainVision Analyzer software (Version 2.1, Brainproducts, Munich, Germany) and subsequently exported to Excel (MS office 2013) and SPSS version 22 (IBM SPSS Statistics).
EEG analysis to explore vigilance state in each pre pulse period consisted of analysing frequency band information. Initially, we planned to perform analysis of vigilance state using the BrainVision Analyzer tool Vigilance Algorithm Leipzig 2.0 (Vigall). To understand why this approach did not turn out to be feasible, a brief description of the Vigall algorithm and its function are given.

Vigall
According to the developers of Vigall, the following vigilance stages can be observed during the transition from high alertness to relaxed wakefulness to drowsiness and finally sleep onset [28]:

- Stage 0: desynchronized non-alpha EEG in the absence of slow horizontal eye movements; found during an activated state (e.g. mental effort)
- Stage A1: with dominant alpha activity corresponding to relaxed wakefulness
- Stage A2: with spreading of alpha activity from occipital to more anterior cortices
- Stage A3: with pronounced frontal alpha activity; beginning of drowsiness
- Stage B2/B3: non-alpha EEG with predominant theta/delta activity, occasional occurrence of vertex waves; drowsiness and transition to sleep onset
- Stage C: commencing with occurrence of sleep spindles of k-complexes; sleep onset

However, due to our interest in segments prior to each TMS pulse disruption, the EEG became discontinuous and this algorithm did not perform optimally. Vigall requires a continuous recording of resting state EEG for at least 15 minutes. Therefore, it was decided to do manual vigilance state analysis using tools available in BrainVision Analyzer.

To extract information solely from the pre pulse periods, pre-processing of the data was necessary.
First the EEG was filtered using a high pass filter with a cut-off frequency of 3 Hz, a low pass filter of 70 Hz and a notch filter. This filtering was performed to remove movement artefacts and other deviations from baseline from the EEG signal and to allow automatic identification of stimulation artefacts. Now, only a (somewhat flattened) EEG signal is left containing very high and narrow TMS pulse artefacts (see figure 5). These high and narrow artefacts were easily identified using a level trigger. 'TMSpulse' markers were put on each EEG channel for each artefact using a value of 100.0 for the threshold and a reset value of 70.0 as estimated from the F8 channel.

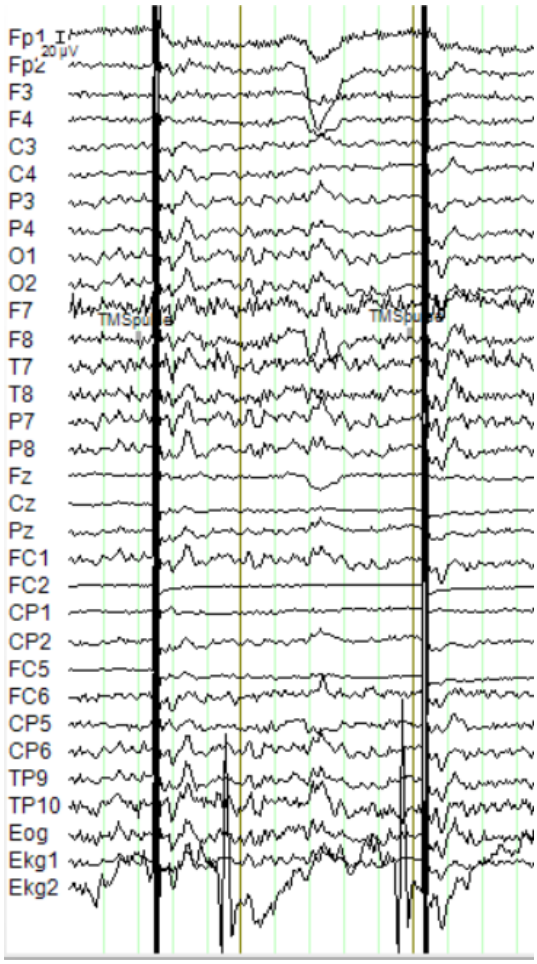

Figure 5: illustration of TMS pulse artefacts in the recorded EEG

This illustration is extracted from BrainVision Analyzer after 'TMSpulse' marker import. No other processing steps influenced the displayed EEG signal. Note the height and narrowness of the artefact, and how it immediately returns to EEG baseline after stimulation.

Since markers were placed in a signal in which almost the entire delta band was filtered out, markers needed to be exported from this signal and reimported into the raw data. After importing the markers in the raw data, filtering was performed again. However, now filtering was minimal to only filter out the slow movement artefacts and to leave the delta band untouched. Thus, data was high pass filtered with a cut-off frequency of 0.5 Hz, as well as low pass filtered with a cut-off frequency of 70 Hz, and a notch filter of 50 Hz was implemented to remove noise.

The next step in data analysis was to obtain the pre pulse segments. To this end, first data was subdivided into the different tasks by manual segmentation based on specific datapoints. Thereupon pre pulse segments of 1400 ms were obtained using segmentation based on marker position.
To extract information regarding different brain rhythms (with different dominant frequencies), the signal needed to be transformed into the frequency domain. So Fourier transformations (FFTs) were performed to evaluate the different pre pulse segments in the frequency domain. This enabled export of all frequency bands of interest representing different brain rhythms, i.e., different levels of attention, to proceed with further analysis in SPSS and Excel.

The following frequency bands were exported:

- delta (1-4 Hz)
- theta1 (4-6 Hz)

- theta2 (6-8 Hz)
- alpha (8-13 Hz)
- beta (13-30 Hz)

According to Vinogradova (1995) dominant activity in the theta 2 band represents a state of hyper attentiveness facilitated in the hippocampus and related structures [74]. For this reason, it was decided to subdivide the theta band into theta 1 and theta 2. Furthermore, Oken et al. (2006) describes a relation between increased beta activity and sustained attention [49]. Therefore, when increased attentiveness is assumed to have a positive impact on MEP amplitude, correlations should be found between the theta 2 and the beta band, and higher MEP amplitudes.
In order to minimize disturbances in the exported frequency bands, EEG electrodes located more laterally on the head were excluded from analysis.

According to Hammond (2003) alpha rhythm is predominantly present in the occipital and parietal regions; beta is mostly found in the frontal and central regions; dominant theta rhythm can be found in the central and parietal regions; whereas delta is mostly found frontally and around the midline [26]. Based on this data, electrodes were selected from which specific frequency band powers were extracted.

The selected electrodes can be observed in table 1:

Table 1: selected frequency bands for extracting power information (μV^2) of the brain rhythms

Frequency band	**Electrodes**
Alpha	O_1 O_2 P_Z
Beta	F_Z C_Z F_3 F_4
Theta	C_3 C_4 C_Z P_Z
Delta	F_3 F_4 F_Z C_Z P_Z

5.4.2 EMG analysis

Acquired MEPs were re-evaluated using Synergy; MEP amplitudes were analysed by marker placement and subsequently transferred to Excel. An overview of the procedure as performed in MEP analysis is described in the following paragraphs.

To obtain amplitude information of each measured MEP, MEPs were re-evaluated in Synergy. Here, using the function "Marker placement" under "Analysis", markers were placed on each MEP to calculate the amplitude. Placement of the markers is illustrated in figure 6. MEP amplitude was calculated as the difference in amplitude between markers 2 and 4. In measurements with double pulse and triple pulse stimulation, markers were placed on the last acquired MEP.

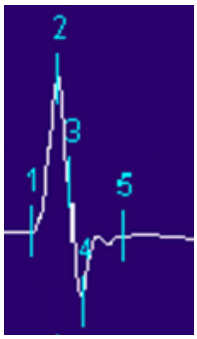

Figure 6: illustration of marker placement for the calculation of MEP amplitude
MEP amplitude was calculated between markers 2 and 4.

To determine which of the five paradigms resulted in MEPs with the lowest intraindividual variability, the coefficient of variance (CoV) was calculated using MS office Excel. The CoV was chosen because it relates the mean MEP amplitude to the MEP standard deviation. In this manner the larger variability in the paradigms that resulted in generally larger MEPs could be taken into account. CoV is calculated as follows:

$$CoV = \sigma/\mu. \tag{6}$$

Here σ is the standard deviation and μ is the mean amplitude of the 20 MEPs acquired for each paradigm per participant.

The effect of excluding outliers of the acquired MEP amplitudes has been explored as well. To do so, first the two main outliers, i.e., the highest amplitude and the lowest amplitude were excluded (now only 90% of the obtained amplitudes are left, hence 'CoV 90%').
This procedure was followed for the exclusion of the four main outliers (two highest and two lowest amplitudes) as well to obtain CoV 80%.

Moreover, due to high intraindividual MEP amplitude variability, it is difficult to compare MEP amplitudes between participants.
To see whether the triple pulse in general leads to higher MEP amplitudes than the double pulse paradigm, it was decided to calculate the ratios between the mean amplitudes (without the two main outliers obtained from double pulse stimulation versus triple pulse stimulation. For this purpose, the ratios the amplitudes of the double pulse stimulation task and the triple pulse stimulation task were calculated for each participant using the following equation.

$$Ratio = \frac{\bar{A}_{triplepulse}}{\bar{A}_{doublepulse}} \tag{7}$$

Here, $\bar{A}_{triplepulse}$ is the mean MEP amplitude obtained during triple pulse stimulation and $\bar{A}_{doublepulse}$ is the mean MEP amplitude obtained during double pulse stimulation.

5.5 Statistical Analysis

5.5.1 Vigilance state

To investigate the relationship between the rhythm information obtained from each pre pulse segment and their accompanying MEP amplitude, correlation analysis was performed.
After extracting the power of each rhythm in each pre pulse segment from the relevant EEG channels, the mean power per frequency band per task for each pre pulse period could be calculated. This provided

20 pre pulse power values for each rhythm for each task per study participant. In each participant, 20 MEP amplitudes were simultaneously acquired for each task. In order to investigate whether a significant correlation exists between power of the different brain rhythms and the simultaneously recorded MEP amplitude, Pearson's correlation tests were performed for each of the pre pulse period rhythm powers per task with the associated MEP amplitudes. To verify normality of the distributions, first Shapiro-Wilk normality tests were conducted. In cases where data were not normally distributed, the nonparametric Kendall's-tau correlation test was performed. A significance level of $\alpha = 0.05$ was adopted.

5.5.2 MEP amplitudes

To see whether a significant decrease in MEP amplitude variability was obtained through use different stimulation paradigms, one-way ANOVAs were performed. To verify normality of the distributions, first Shapiro-Wilk normality tests were conducted. Next, one-way ANOVAs were performed between the different tasks per participant and their accompanying CoVs 80% and CoVs 90%. Thereafter, the Bonferroni corrected test was done to investigate specifically between which of the paradigms a significant decrease in variability was present. A significance level of $\alpha = 0.05$ was adopted.

6 Results

6.1 Healthy participants

6.1.1 Vigilance state

For illustration purposes, figure 7 represents scatterplots of specific mean rhythm power (μV^2) per pre pulse period (delta, theta1, theta2, alpha, and beta, respectively) versus the simultaneously recorded MEP amplitude (mV) in a single participant during the triple pulse task.

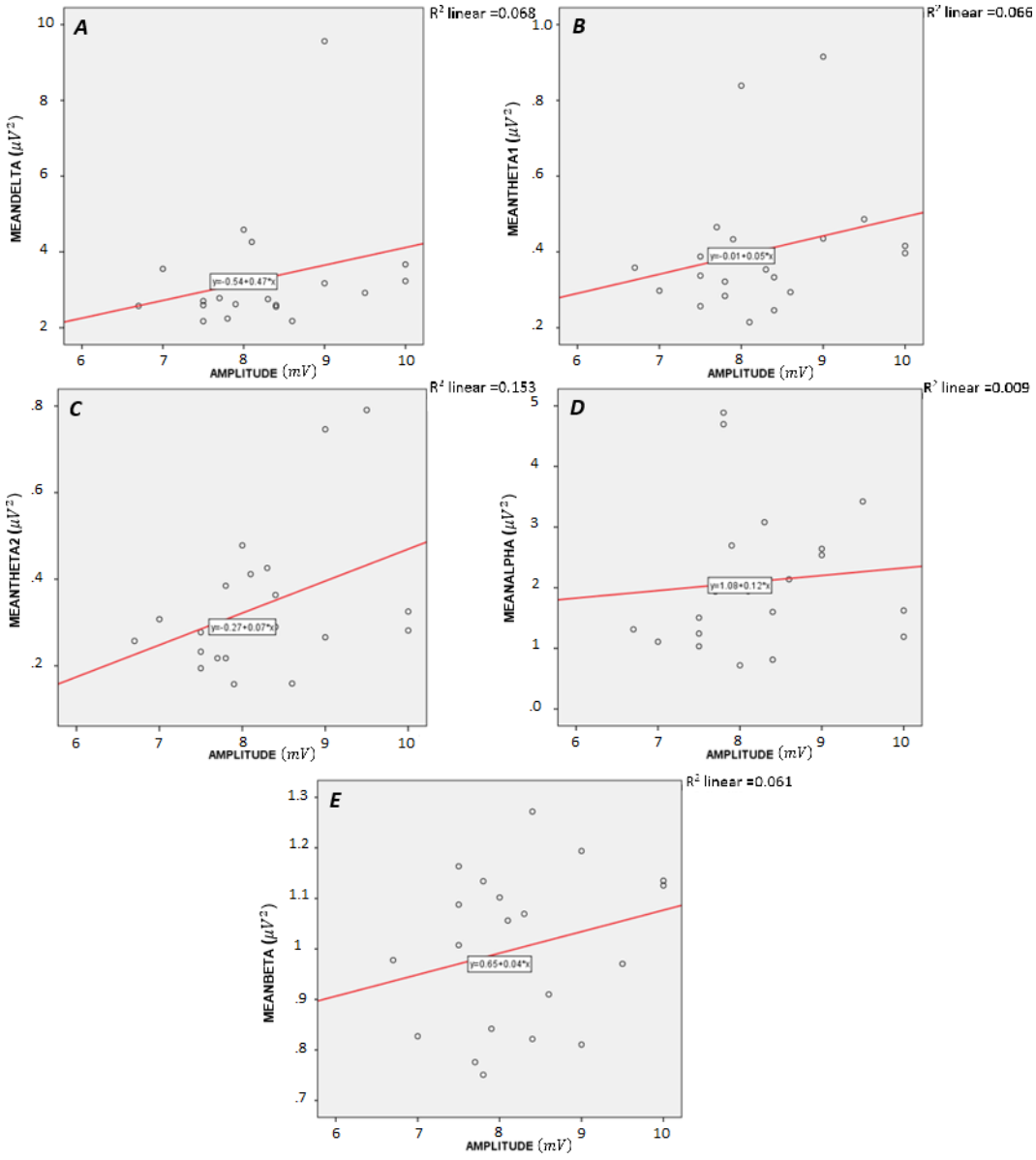

Figure 7: scatter plots of the brain rhythm powers (μV^2) averaged over the chosen electrodes as described in table 1 plotted against the mean amplitudes (mV) per pulse measured during the triple pulse task in a single healthy participant

Part A represents the mean pre pulse delta power plotted against the simultaneously recorded amplitude. Part B and C display the mean pre pulse power in the theta 1 and theta 2 band, respectively, plotted against the simultaneously recorded amplitude. Part D shows the mean alpha power plotted against the simultaneously acquired MEP amplitude. Part E represents the mean pre pulse beta power versus the simultaneously obtained MEP amplitude. Please note the scaling differences between the scatterplots.
A higher level of attention would have corresponded to a decrease in theta1 power and an increase in theta2 and beta power.

Please note the scaling differences between the scatterplots in figure 7 due to different powers and amplitudes. For a brief overview, slope formulas derived from the linear lines in the scatter plots and the coefficients of determination (R^2) are provided in table 2.

Table 2: an overview of the slope formulas of the linear regression lines and the coefficients of determination of the scatterplots in figure 7

Frequency band	**Slope formulas**	**Coefficients of determination (R^2)**
Mean delta vs. amplitude	0.54+0.47x	0.068
Mean theta1 vs. amplitude	0.01+0.05x	0.066
Mean theta2 vs. amplitude	0.27+0.07x	0.153
Mean alpha vs. amplitude	1.08+0.12x	0.009
Mean beta vs. amplitude	0.65+0.04x	0.061

Pearson's correlation tests have been performed for each participant, however results were non-significant; thus, a significant correlation between the presence of a specific brain rhythm and a larger MEP amplitude could not be found.

6.1.2 MEP amplitude

A total of twenty healthy participants (the first five participated in the pilot study, the other 15 took part in the main study) recruited from relatives, staff members of the UMCG or via advertisements, were included in this study. They had no prior neurological disease and were not on medication influencing cortical excitability (as confirmed by questionnaires taken prior to each measurement). They were 6 men and 14 women ranging from 22 to 56 years of age.

To illustrate the results, first examples of acquired MEPs in one participant obtained consecutively during a single measurement session are shown and discussed (figure 8).

Part A of figure 8 shows MEPs obtained during the first task of the measurement, in which the participant was relaxed. Even in this short sequence, intraindividual variability in MEP amplitude is clearly visible.

Part B shows MEPs obtained during the second task, the contralateral precontraction task. As in part A of this figure, intraindividual amplitude variability between the MEPs is clearly visible, even though there is minor overlap between the MEPs. .

Part C and D show MEPs obtained while the participant was performing the tale reading task and undergoing the double pulse stimulation task, respectively; these were the third and fourth tasks of the measurement session. Variability in the obtained MEPs itself is difficult to see in parts C and D, however, a difference between oscillatory patterns post-MEP can be observed; a difference in oscillatory amplitude can be seen, also the consecutive oscillations are not synchronized.

In the last part of figure 8, part E, MEPs obtained during triple pulse stimulation in a single measurement session are shown. Here, intraindividual MEP variability is visibly less as can be seen in the visible parts from the consecutive MEPs; even oscillations following the motor responses are of the same shape. In addition, please note the increase in MEP amplitude from the first task (6A) through the fifth task (6E).

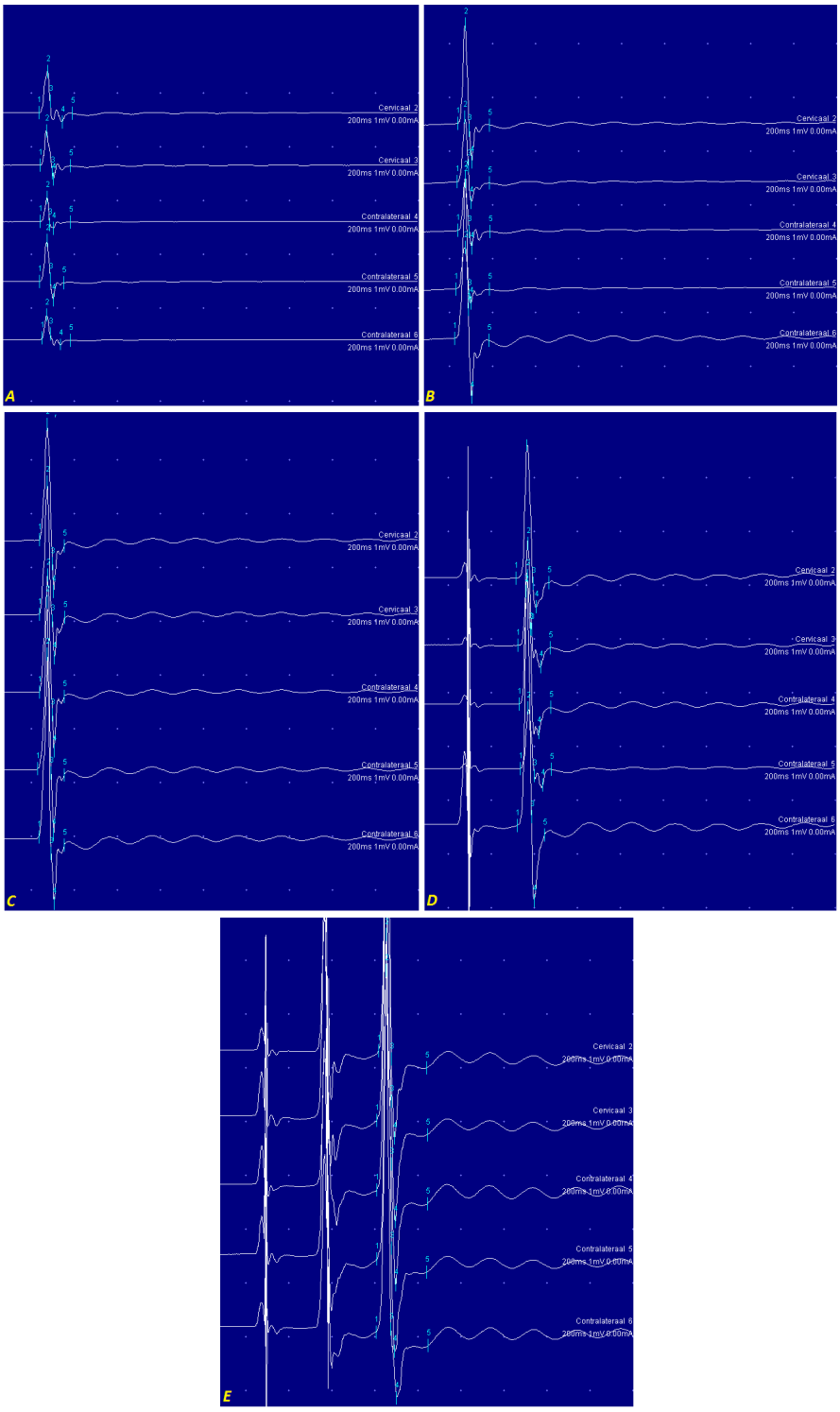

Figure 8: an example of obtained MEPs

This figure shows obtained MEPs during the single pulse relaxed task (A), the contralateral precontraction task (B), the tale reading task (C), the double pulse stimulation task (D), and the triple pulse stimulation task (E). Even in this short sequence of consecutively measured MEPs, clear variability in amplitude is visible in parts A and B. When observing post MEP oscillations, variability is seen in parts C and D as well. The least MEP amplitude variability can be observed in MEPs obtained with triple pulse stimulation.

For a better comparison between the different amplitudes that were obtained, a MEP obtained with the single pulse relaxed paradigm and a MEP obtained with the triple pulse stimulation paradigm are shown in figure 9.

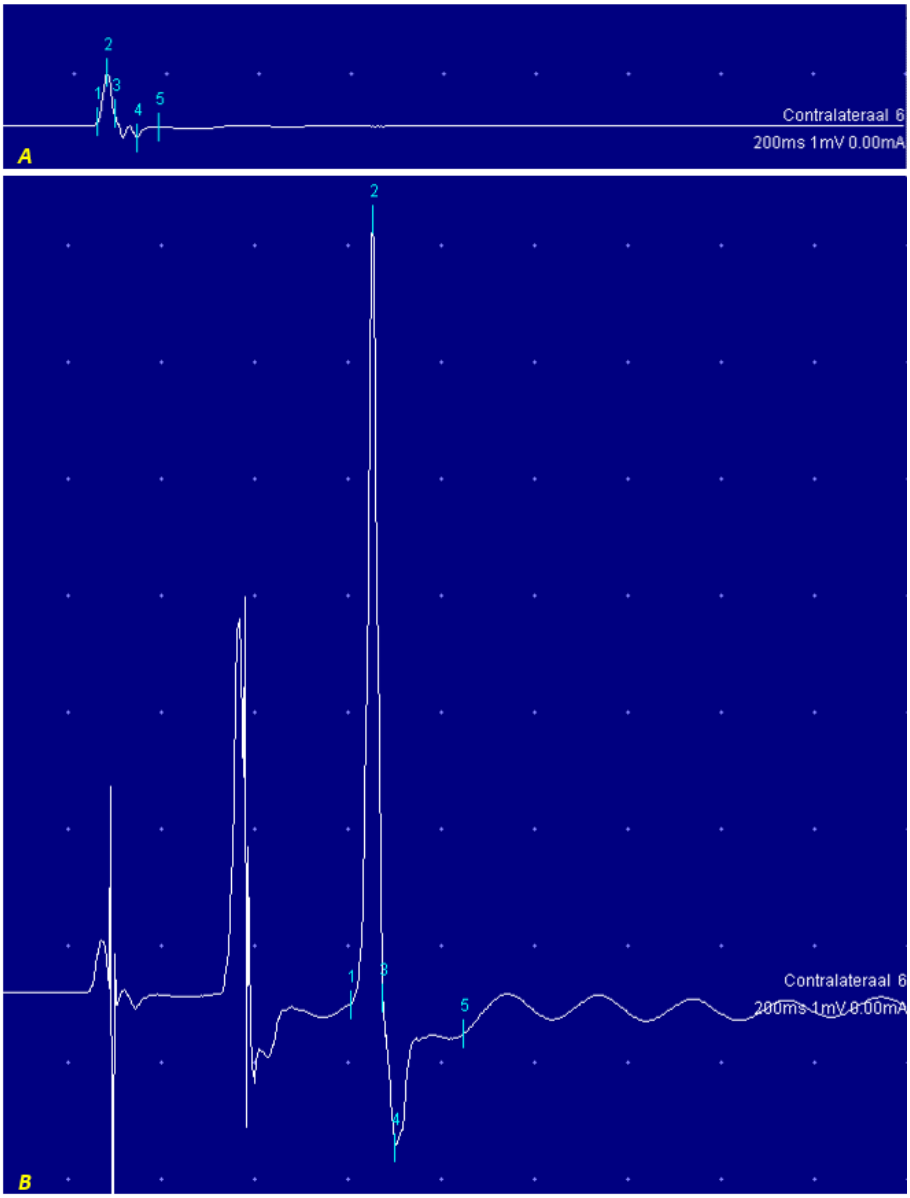

Figure 9: impression of the difference in MEP amplitude between the first task (single pulse stimulation during relaxation) (A) and the fifth task (triple pulse TMS)(B)
A clear difference in amplitude is shown. Note that with each pulse in the triple pulse stimulation amplitude increases; thus a facilitatory effect due to a decrease of the motor threshold by the previous evoked MEP amplitude occurs with acquisition of the following MEP.

To provide a clear presentation of the CoVs, boxplots were made. In figures 10 and 11, the different paradigms are shown on the horizontal axes plotted against the CoVs on the vertical axes.

In figure 10, main outliers were excluded from the graph (CoV 90%). Here, it is shown that the triple pulse stimulation paradigm has the lowest CoV.

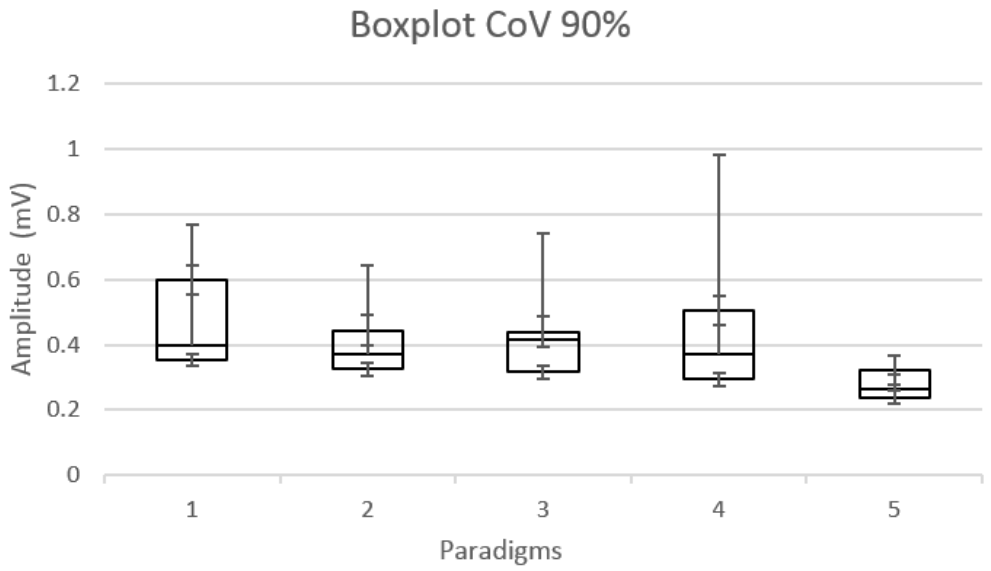

Figure 10: boxplot of CoV 90% of the measured MEP amplitudes in healthy participants
1: single pulse relaxed task; 2: contralateral precontraction task; 3: tale reading task; 4: double pulse task; 5: triple pulse task. This figure displays the least variance in amplitude (regarding the mean amplitude) when using the triple pulse stimulation paradigm.

Subsequently, the two highest and two lowest amplitudes in each measurement were excluded, to calculate CoV 80%. The resulting boxplots can be observed in figure 11. Here, a visible enforcement of the reduction in CoV resulting from stimulation using the triple pulse stimulation paradigm compared to figure 10 can be observed; when excluding the two highest and lowest outliers, the CoV of the triple pulse stimulation paradigm decreases even more.
For an overview of the values of the mean amplitudes, standard deviations and calculated CoVs used to plot figures 10 and 11, please refer to Appendix B.

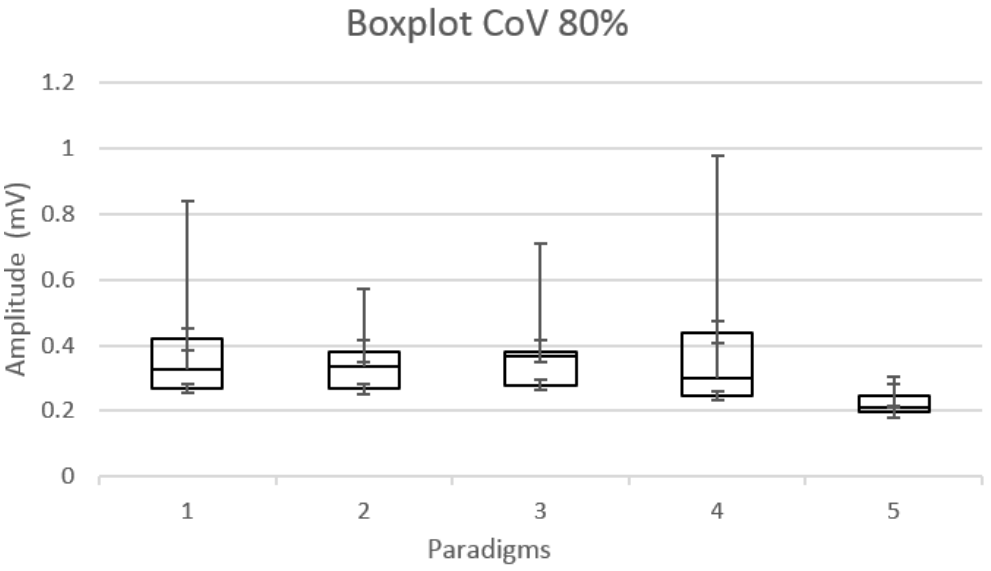

Figure 11: boxplot of CoV 80% of the measured MEP amplitude in healthy participants
1: single pulse relaxed task; 2: contralateral precontraction task; 3: tale reading task; 4: double pulse task; 5: triple pulse task. The lowest CoV is observed for the triple pulse paradigm.

One-way ANOVAs have been performed to see whether a significant difference in MEP amplitude variability exists between the different stimulation paradigms. Outcomes of one-way ANOVAs confirmed that a significant difference in MEP amplitude variability between tasks exists ($p = 0.009$) based on cal-

culations of CoVs 90%. A significant difference in MEP variability between tasks based on calculations of CoVs 80% was not confirmed. Outcomes of the one-way ANOVA are shown in figure12.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cov90	Between Groups	.427	4	.107	3.705	.009
	Within Groups	2.018	70	.029		
	Total	2.445	74			
cov80	Between Groups	.264	4	.066	2.295	.068
	Within Groups	2.013	70	.029		
	Total	2.277	74			

Figure 12: outcome of one-way ANOVA

Results of the subsequently conducted post-hoc Bonferroni test showed that a significant difference exists ($p = 0.005$) between MEP variability due to stimulation with the single pulse relaxed paradigm and stimulation with the triple pulse paradigm. Thus, MEP amplitude variability is significantly decreased by using the triple pulse stimulation paradigm compared to using the single pulse stimulation paradigm. Outcomes of the Bonferroni corrected test are shown in figure 13.

Bonferroni

Dependent Variable	(I) taak	(J) taak	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
cov90	1.00	2.00	.07740	.06200	1.000	-.1023	.2571
		3.00	.08075	.06200	1.000	-.0990	.2605
		4.00	.05768	.06200	1.000	-.1220	.2374
		5.00	.22821*	.06200	.005	.0485	.4079
	2.00	1.00	-.07740	.06200	1.000	-.2571	.1023
		3.00	.00335	.06200	1.000	-.1764	.1831
		4.00	-.01972	.06200	1.000	-.1994	.1600
		5.00	.15082	.06200	.176	-.0289	.3305
	3.00	1.00	-.08075	.06200	1.000	-.2605	.0990
		2.00	-.00335	.06200	1.000	-.1831	.1764
		4.00	-.02307	.06200	1.000	-.2028	.1567
		5.00	.14747	.06200	.201	-.0323	.3272
	4.00	1.00	-.05768	.06200	1.000	-.2374	.1220
		2.00	.01972	.06200	1.000	-.1600	.1994
		3.00	.02307	.06200	1.000	-.1567	.2028
		5.00	.17054	.06200	.076	-.0092	.3503
	5.00	1.00	-.22821*	.06200	.005	-.4079	-.0485
		2.00	-.15082	.06200	.176	-.3305	.0289
		3.00	-.14747	.06200	.201	-.3272	.0323
		4.00	-.17054	.06200	.076	-.3503	.0092

Figure 13: outcome of the Bonferroni post-hoc test

Furthermore, MEP amplitudes were compared between individuals to see whether triple pulse stimulation, in general, leads to higher MEP amplitudes relative to double pulse stimulation. The ratios between the mean amplitudes as obtained with double pulse and triple pulse stimulation (without the two main outliers) for each participant have been calculated and are shown in figure 14. The CoVs are also plotted in this figure to be able to see whether a high ratio value is accompanied by a high intraindividual variability.

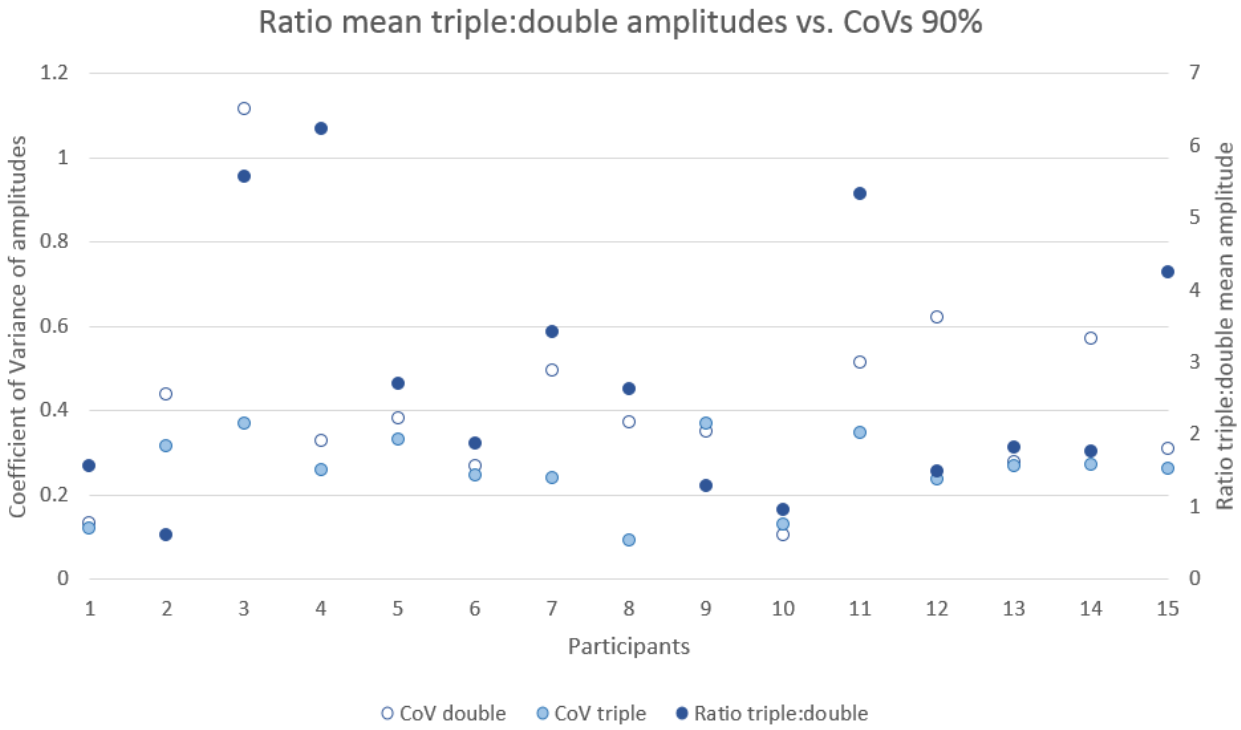

Figure 14: boxplot showing the ratios between double pulse and triple pulse mean MEP amplitudes per participant plotted against the CoVs of double and triple pulse stimulation MEP amplitudes per participant

Please note the double vertical scale, one on the left for the values of the CoVs and the right scale for the ratio values.

From figure 14 it can be concluded that in most participants, the triple pulse CoV is lower than the double pulse CoV, indicating that also on an individual level, the lowest intraindividual variability is obtained during triple pulse stimulation. From observing the ratio values, the opposite can be said regarding interindividual variability; a broad distribution of triple:double mean amplitude ratio values is shown indicative of a high interindividual variability. In addition, (nearly) all ratio values are >1. So in (nearly) every participant the absolute amplitude increases during triple pulse stimulation.

6.2 Results: stroke patients

Results obtained in healthy participants showed that a significant decrease in MEP amplitude variability can be achieved through using the triple pulse stimulation paradigm. This paradigm was therefore chosen as the "optimal" stimulation paradigm for subsequent feasibility testing in stroke patients.

Two stroke patients with a partial motor deficit in the upper extremity resulting from first ever ischaemic stroke were included. They were investigated with TMS within 72 hours after stroke onset. One of the patients was a female of 81 years old (referred to as patient 1 from here on) with a known history of diabetes mellitus. Electrocardiography (ECG) confirmed the presence of atrial arrhythmia. The other patient was a male of 80 years old (referred to as patient 2 from here on) with a known history of aortic stenosis.

The diagnosis of stroke was made by clinical examination and haemorrhagic stroke was excluded by means of a CT scan. Patient 1 was diagnosed with an ischaemic CVA in the right hemisphere, whereas the diagnosis of patient 2 was an ischaemic CVA in the left hemisphere. The cerebral CT of patient 2 additionally showed two small lacunar infarctions in the lentiform nucleus; the lesion sizes were not measured.

Further examination by means of duplex ultrasonography showed no significant stenosis of the carotid arteries in patient 1, however in patient 2 based on blood flow velocities a stenosis between 50-70% was found. In both patients, moderate to severe atheromatous vascular changes were seen on ultrasound examination.
None of the patients had a history of prior neurological disease. They received rehabilitating and appropriate medical treatments, but no drugs interfering with excitability.

Obtained MEP amplitudes in patient 1 and 2 are shown in figures 15 and 16, respectively. The upper part of figures 15 and 16 (A) contains MEPs evoked with single pulse stimulation (for finding the rMT), the lower part (B) contains MEPs elicited with triple pulse stimulation. Patient 1 was stimulated at a stimulation intensity of 70% of maximum stimulator intensity, and patient 2 was stimulated at a stimulation intensity of 65%. Both patients were relaxed and comfortable throughout the entire measurement and mentioned they did not perceive the experiment as demanding at all. Furthermore, both patients were able to be transported per wheelchair and were considering their recent CVA in fairly good condition.

The MEPs acquired with triple pulse in patient 2 are very different from the clear MEPs acquired with the same paradigm in patient 1. Nearly no muscle response is seen in figure 16 part B.

To decide whether this paradigm is feasible to apply in stroke patients, first the definition of feasibility should be determined. According to the Cambridge advanced learner's dictionary, feasibility is: "an examination of a situation to decide if a suggested method, plan, or piece of work is possible or reasonable" [1].
For this study it was decided to regard feasibility as the examination of the applicability of the triple pulse stimulation paradigm. In addition, the triple pulse stimulation paradigm would only be regarded feasible if MEPs were obtained.

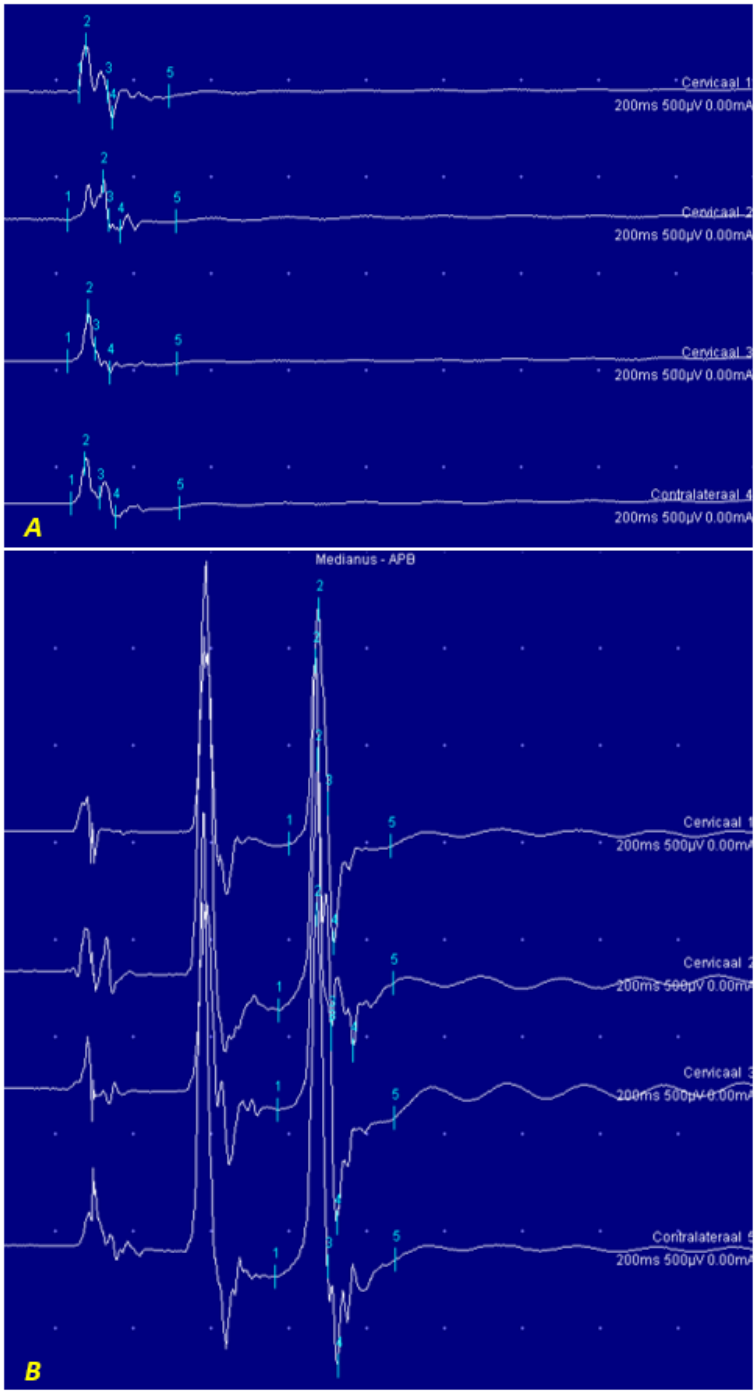

Figure 15: examples of MEPs obtained during measurement in patient 1

The upper part (A) contains MEPs that were elicited using the single pulse TMS paradigm, the lower part (B) shows MEPs obtained during triple pulse stimulation. Clear consecutive MEPs consistent in amplitude were obtained with triple pulse stimulation.

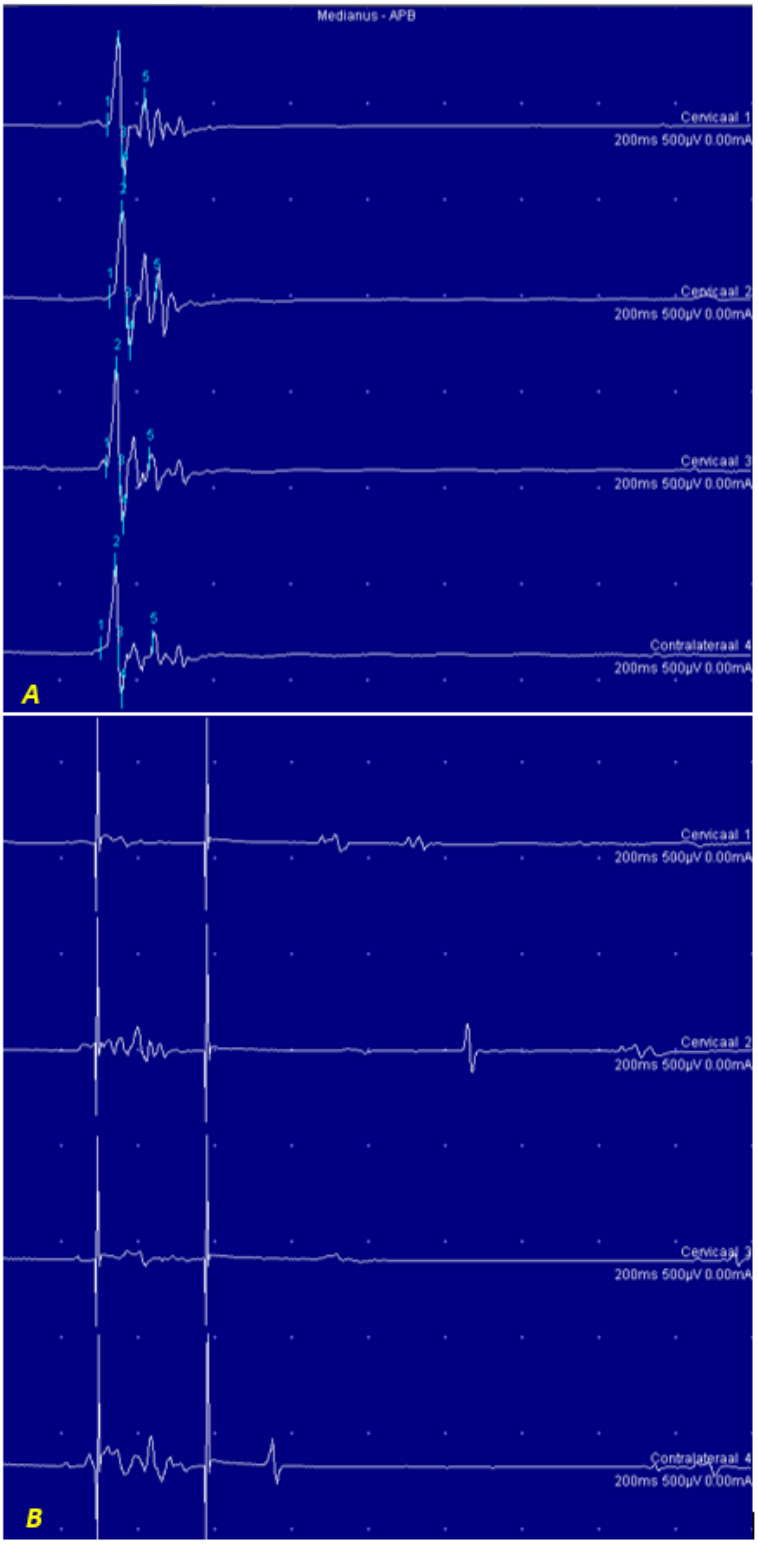

Figure 16: examples of MEPs obtained during measurement in patient 2

The upper part (A) contains MEPs that were elicited using the single pulse TMS paradigm, the lower part (B) shows MEPs obtained during triple pulse stimulation. Nearly no MEPs were obtained during the consecutively admitted triple pulse stimulation; an inhibitory effect is suspected.

7 Discussion

In this study we investigated the influence of vigilance state on MEP amplitude in healthy participants while they were executing five different tasks. We found no relation between vigilance state, as determined from the EEG signal preceding TMS, and MEP amplitude during either of the tasks. However, intraindividual MEP amplitude variability did differ between tasks although this did apparently not depend on vigilance state; minimum variability was obtained during triple pulse stimulation. As a secondary aim of this study was to minimize MEP variability to increase its reliability as a predictor for outcome in stroke, the feasibility of the triple pulse paradigm was tested in stroke patients.

7.1 Influence of vigilance state

7.1.1 Tasks influencing vigilance state

There are at least three possible causes for the absence of significant correlations between EEG based pre-MEP vigilance state and MEP amplitude during either of the tasks. First, it could be that the conditions in which the different tasks were performed did not affect vigilance state (strongly) enough; i.e., all tasks were conducted in the same basal conditions namely wake, not after sleep deprivation. However, we did not mean to influence vigilance state in order to see its effect on MEP amplitude. The aim was to investigate whether vigilance under normal conditions would affect MEP amplitude significantly. Whether extreme variations in vigilance state influence MEP amplitude could be proposed as a new research question for another study.
Possible ideas for a future study could be to measure MEP amplitude in different conditions, for instance in basal conditions, partial sleep deprivation, and sleep. In a study where dynamic changes in spontaneous EEG rhythms under influence of vigilance states were investigated, these different conditions were included [43]. They performed the first measurement in basal conditions, the next session was performed the day after partial sleep deprivation for which the patients were instructed to stay awake from 3 am until morning (no napping permitted). The last measurement session immediately followed the second session where they allowed the participant to sleep. These conditions resulted in a pronounced effect of vigilance state as they found a significant theta synchronizing effect over the posterior cortical areas [43]. Increased theta in turn is often correlated with a lower level of attentiveness [49].
Another argument for incorporating different conditions to induce a larger influential effect from vigilance state is that prolonged wakefulness has been described to increase cortical excitability [31]. Huber et al. (2013) found that prolonged wakefulness produces a significant increase in the amplitude and slope of the early local field potential response to cortical stimulation. This increased cortical excitability could in turn increase MEP amplitude [57] and may be interesting to investigate.

7.1.2 Use of Vigall

Another possible explanation for the lack of significant correlations between the brain rhythms studied and the simultaneously obtained MEP amplitudes could be analysis of discontinuous EEG. Stimulation artefacts induced by TMS provided excellent thresholds for marker position to process only a selected time-frame prior to the TMS pulse. However, this resulted in a discontinuous EEG making the use of Vigall impossible; Vigall requires a resting state continuous EEG period of at least 15 minutes [28]. A solution to this problem could be to record resting state EEG for 15 minutes prior to initiating the first task; however, this increases measurement session time.
In addition, when a continuously measured EEG would have been available, Vigall may not have performed optimally either since the algorithm incorporates independent component analysis (ICA) based on horizontal electro-oculogram (HEOG) recordings [28]. This specific recording was not included in our study.

A point of discussion that may lead to dissapointing results, even when using Vigall is that because of the short intervals in which vigilance state is defined, it is expected to see some fluctuation between two stages but no major differences between different tasks in one participant. This analysis method could prove not to possess the means to show the influence of a specific brain rhythm on MEP amplitude; i.e., to see how a rhythm's power in a single pre pulse segment correlates to simultaneously recorded MEP amplitudes.

7.1.3 Delta and theta band interference

The absence of a significant correlation between the simultaneously recorded pre pulse brain rhythms and MEPs could also be due to disturbances in the EEG signal by limb movement induced by TMS. Participants were instructed to relax and keep their eyes open during the first task, but movement induced by TMS startled some of them, inducing more movement. Indeed large deflections in the EEG were observed post stimulus which partly interfered with the subsequent pre pulse period.
In addition, upon double pulse stimulation, limb movement increased. Furthermore, during double and triple pulse stimulation, most participants could not contain their laughter, inducing even more movement.

These phenomena gave rise to ideas about how the frequency distribution would look like in the latter two tasks, double and triple pulse. Movement artefacts in the EEG exist in the lower frequency bands, delta and theta. Due to their dominant presence in most participants, they possibly interfere with determining how much influence different vigilance states have on MEP amplitude variability. For these reasons, we expected the delta and theta bands to dominate the EEG during these tasks due to slow movements observed in the EEG caused by limb movement and laughter. In general, patients were very tensed and unfamiliar with TMS. This generalisation excludes one of the healthy participants. This participant was already familiar with TMS research and was able to relax very well. Therefore, to explore the hypothesis as mentioned before, the mean amplitudes together with the mean brain rhythms were plotted for each task for a tensed participant and subsequently for the relaxed participant (figures 17 and 18, respectively).

Figure 17 shows a notable increase in delta and theta 1 during the double pulse task. The data shown in figure 17 was obtained from a single tensed participant. This graph provides an indication of the outlook of data acquired in most participants, since nearly all had trouble relaxing and were startled by magnetic stimulation. Figure 18 shows data obtained from a single relaxed participant, already familiar with TMS research.

In the relaxed participant a slight increase in all rhythms is accompanied by a rise in MEP amplitude. This observation lead us to hypothesize that by letting the participants become acquainted to the TMS sensation prior to recording, the EEG disturbance of the delta and theta frequency bands could be avoided in future research. Due to time constraints, because of the long-duration of the measurements in this study, it was not possible to let the participants get fully used to TMS. In future studies, perhaps by incorporating fewer tasks, this should be considered. Another possible solution for this problem could be to only include healthy participants already familiar with TMS research.
Important to note: data used for plotting figures 7 and 18 were obtained from the same participant.

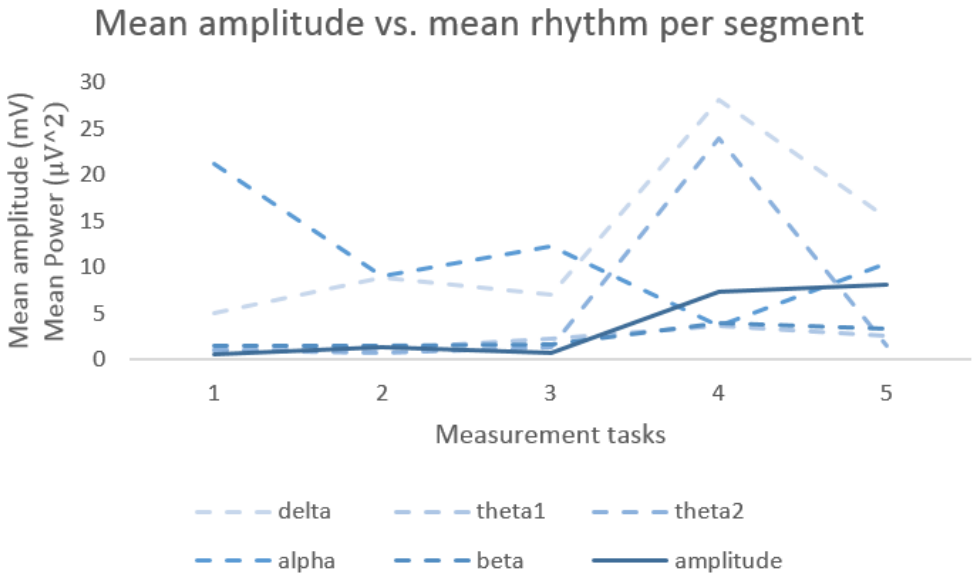

Figure 17: comparison of the mean MEP amplitude (mV) per task and the mean power (μV^2) for each brain rhythm of interest in a tensed participant

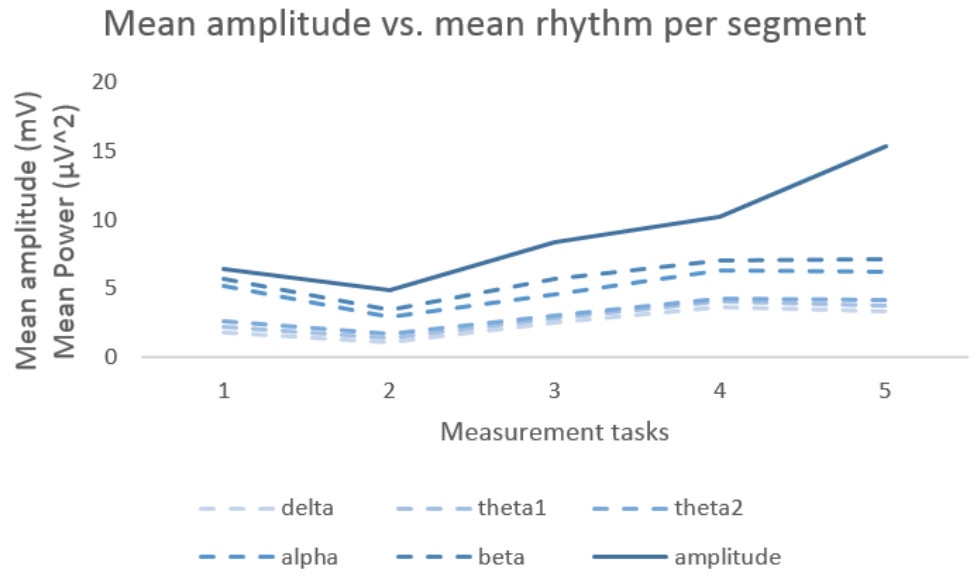

Figure 18: comparison of the mean MEP amplitude (mV) per task and the mean power (μV^2) for each brain rhythm of interest in a relaxed participant

7.2 MEP amplitude variability

The lowest MEP amplitude variability as expressed in its CoV, was obtained during the triple pulse task. An additional advantage of this task was that MEPs were most easily obtained and had the largest amplitudes compared to the other four stimulation paradigms.

Amplitudes obtained during the contralateral contraction task were very variable due to movement of the participants while making a fist. In addition, the examiner had to cue the participant to make a fist and this also hindered keeping the coil as still as possible.

Furthermore, during the double pulse stimulation and triple pulse stimulation, coil movement was caused by TMS induced movements of the participants. Coil movement should be taken into consideration when interpreting the results based on the acquired amplitudes as this may have contributed to the

variability [34, 36]. Although neuronavigation enabled recovery of the location and orientation of the coil, differences in orientation between the stored information and the current coil position was difficult to determine visually. Therefore, we cannot be entirely sure of recovery of the exact same coil position throughout the double pulse and triple pulse tasks.
In addition, all measurements may be influenced by respiratory artefacts always causing slight movement in the participants. In future research, to avoid EEG disturbances due to any kind of movement it should be considered to place the participants' head in a fixed position to avoid coil displacement.

7.3 Feasibility in patients

7.3.1 Location of ischaemic stroke

Between the two included patients, there were minor differences in the inconvenience they experienced following their stroke. This may have lead to smooth conduction of the experiments. In addition, they did not perceive the measurements as burdensome at all and were happy to comply.
Unfortunately, results between the two patients included in the feasibility study were very divergent. In one patient triple pulse stimulation produced, like in healthy participants, reproducible MEPs. In the other patient no MEPs were obtained through triple pulse stimulation. There are at least two possible explanations for the diversity in results. A first explanation for the diversity in results could be the location of the ischaemic infarction. It was thought that abnormal results in triple pulse stimulation could be caused by CMCT abnormalities resulting in unsynchronized firing of motor neurons which would result in never reaching the motor threshold. According to a study on changes in MEP depending on the location of ischaemic stroke, two types of CMCT abnormalities can occur: 1) unrecordable MEP by TMS induction, and 2) prolongation of CMCT [44]. The authors described that MEPs are an all or none phenomenon based on the number of surviving neurons and corticospinal tracts. Misra and Kalita (1995) mention subcortical infarctions, like in patient 2 in our study (lacunar infarction), to result in damage or distortions to the descending motor pathways by infarction or oedema. If the motor pathways are sufficiently damaged, prolongation of CMCT occurs which leads to desynchronized motor neuron firing and ultimately a loss of MEP [44]. However, this still does not answer the question why in our study only during triple pulse stimulation an absence of MEPs occurred and not when stimulating according to the relaxed single pulse stimulation paradigm. Therefore, other possible causes were also explored.

7.3.2 Increased intracortical inhibition

Another explanation for the diversity in obtained results among patients is that from post-measurement checking of the patient history and observing radiological reports based on a recently acquired CT scan, older ischaemic lesions were found in the basal ganglia in patient 2. These findings were not mentioned in the general patient history.
In a recent study the mechanism of cortical reorganization was expected to have great influence in recovery after stroke [30]. Honaga et al (2013) state that there is still little information about short intracortical inhibition in the chronic phase (over six months from stroke onset) after stroke and they mention that the inhibitory circuit in the primary motor cortex plays an important role in functional reorganization. They showed that increased intracortical inhibition was observed in stroke patients with severe hemiparesis.
Furthermore, in pre-activated muscles, TMS induces a transient suppression of EMG after the elicited MEP (this transient suppression, or inhibitory effect, is commonly referred to as the silent period) [69]. In a study where silent period alterations due to thalamic stroke were investigated, it is described that thalamic infarction causes prolongation of the cortical silent period [13]. Since basal ganglia are strongly interconnected with the thalamus [32], the inhibitory effect as seen in this study in the second patient might be explained by a prolongation of the silent period. This would explain why single pulse stimulation

does elicit MEPs, but triple pulse stimulation using an interstimulus interval of 28.6 ms would not.

7.3.3 Number of patients

During the set-up of this study, stroke patients were anticipated to be brought into the hospital regularly. Inclusion of at least 15 stroke patients was expected to be no problem at all. However, practice showed the opposite. Stroke patients were admitted to the hospital, however few were eligible for participation in this study. Most common causes of unsuitability were the type of ischaemic stroke and the presence of aphasia. In six weeks reserved for inclusion and measurement of stroke patients, over 40 stroke patients were admitted to the hospital. Patients that at first were thought to be eligible were found to have no ischaemic stroke, but a haemorrhagic stroke. As mentioned above, aphasia was the second most common cause of exclusion. Other reasons for exclusion were: death, dysarthria, unconsciousness and the current stroke not being their first ever stroke. There could have been another inclusion, however this patient suffered from impairment of pulmonary function and decided at the very last minute that he would rather refrain from participating in this study since it would be too much to handle at that moment.
Future research should consider to include patients with haemorrhagic stroke as well since this could increase patient numbers substantially.

7.3.4 Feasibility

Both patients were able to sit upright for transportation per wheelchair and were considering their recent CVA in fairly good condition. Most patients worse affected by stroke could not be included based on the inclusion criteria. Whether the triple pulse paradigm would have been feasible in deteriorated patients transported per bed, could not be tested and is therefore unknown.
Possible difficulties could arise when using neuronavigation with bedridden stroke patients. For instance, the spherical movement sensors mounted on the patient's head by means of an elastic headband need to maintain the exact same position throughout the measurement. During measurements, movement induced by TMS could cause displacement of the headband, and consequently the sensors, due to friction against the pillow.
Furthermore, tubes and wires connected to the patient could propose problems as well. Important to note here is that presence of conductors (metals) when applying TMS should always be taken into account.
Thus, use of neuronavigation could complicate triple pulse stimulation in clinical practice. For future research, it should be considered to refrain from using neuronavigation during measurements in stroke patients, taking into account a slight increase in MEP amplitude variability due to coil displacement.

7.4 Technical Constraints

It is also important to be aware of the technical constraints encountered during the conduction of this study. Technical limitations were for example the presence of a single Magstim magnetic stimulator and the fact that interstimulus interval selection was limited by stimulus intensity.
The consequence of only having a single stimulator available was that we were solely able to stimulate at a certain stimulus intensity during application of a train of consecutive stimuli (like in double and triple pulse stimulation). Previous studies using double pulse stimulation all used two different magnetic stimulators to induce a conditioning subthreshold pulse followed by a suprathreshold test pulse [7, 10, 11, 40, 45, 46, 59, 67, 68, 76, 77]. Because our study used a different approach, results are difficult to compare. The same comparability issue accounts for triple pulse TMS [27, 58, 59].
Furthermore, because interstimulus intervals were limited by stimulus intensity, in some participants the optimal interstimulus interval of 28.6 ms (as determined based on an article by Mark Hallett (2007)) could not be maintained. In these cases, the lowest possible time period between pulses was chosen.

7.5 Neuropathy check

During inclusion of healthy participants in our study, one of the inclusion criteria was that the participants were (perceivably) healthy. However, further examination was not conducted to confirm the absence of underlying neuropathies affecting measurements.

Future research should consider the latency of the measured MEP combined with a measure of the peripheral motor conduction time (PMCT). Through calculation of CMCT by subtracting the peripheral latency from the cortical MEP latency, the pyramidal tract function can be evaluated [75]. In our study, solely cortical stimulation was performed and therefore the pyramidal tract function could not be evaluated.

8 Conclusions

In this study, the influence of vigilance state on MEP amplitude was studied. In addition a TMS stimulation paradigm was developed that minimizes MEP amplitude variability in healthy participants. Subsequently the feasibility of this stimulation paradigm was tested in stroke patients. The main conclusions of this study are:

- MEP amplitude variability is decreased in healthy participants when using a triple pulse stimulation paradigm.
- An influence of vigilance state on MEP amplitude could not be confirmed in this study.
- Unfamiliarity and tenseness during TMS-MEP measurements causing undesired limb movements could have influenced EEG recordings to such an extent that the possible correlation between vigilance and MEP amplitude was overshadowed.
- MEPs are more easily obtained with the triple pulse stimulation paradigm (with an interstimulus interval of 28.6 ms and a triple pulse stimulation intensity of 120%) compared to single pulse stimulation.
- The feasibility of the triple pulse stimulation paradigm in stroke patients could not be confirmed in this study, because of a low number of included patients.

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10 Appendices

10.1 Appendix A

10.1.1 Basic theory of electromagnetism

Two types of electric charge exist: static electric charges and induced electric charges. Static electric charges exert force on each other. As determined by Coulomb in 1784: like charges repulse each other and unlike charges attract [25].

The electric potential describes the properties of the field surrounding static electric charges. This field is a scalar field, i.e., a magnitude defines each point in space. According to the following equation: a charge q, in a region where an electric field $\overrightarrow{E}$ exists, is subject to a force $\overrightarrow{F}$ [25, 61].

$$\overrightarrow{F} - q\overrightarrow{E}. \tag{8}$$

When considering that the electric field points along the x-axis, equation 9 can be rewritten to:

$$F_x - qE_x. \tag{9}$$

Under the influence of this force, a charged particle moves by a distance dx, where E_x can be considered constant.

$$F_x dx \tag{10}$$

Moving electric charge inside a conductor can produce a magnetic field. Large currents flowing in coils (conductors) are the creators of magnetic fields in magnetic stimulation and this is the concept behind the clinically used technique transcranial magnetic stimulation (TMS). This concept will be further elaborated below.

In 1831, Faraday attempted to use static magnetic fields to produce a steady electric current in a loop of wire. He did not succeed, but observed that when a magnet was brought close to the coil or removed from the coil currents did flow momentarily [25]. Here, the magnetic flux near the coil changes with time, inducing an electric field in the coil. This electric field is proportional to the rate of change of the magnetic field, which results in free charges flowing in the coil [19, 25]. This process is described by:

$$\oint \overrightarrow{E} \cdot d\overrightarrow{l} = -\frac{d\Phi_B}{dt}. \tag{11}$$

Here, $\overrightarrow{E}$ is the electric field that does the work of moving the electrons in the wire, $d\overrightarrow{l}$ is the element of displacement along the path of integration, and $-\frac{d\Phi_B}{dt}$ represents the change in magnetic flux with time. This equation relates the changing magnetic flux to the electric field it produces. The integral sign on the left indicates the boundary surrounding the path enclosing the area through which the magnetic flux is changing. The minus sign reflects that currents flow in opposite direction from the change in magnetic flux.

When the flux is written in terms of the magnetic flux density, the following equation is obtained [22, 25]:

$$V = \oint_S \overrightarrow{E} \cdot d\overrightarrow{l} = -\frac{d}{dt}\int_S \overrightarrow{B} \cdot d\overrightarrow{S}. \tag{12}$$

Here, the right integration is over a surface S defined by the coil and V represents the electric potential.

Without movement of the coil, S does not change over time, and the equation can be rewritten to:

$$V = \oint \overrightarrow{E} \cdot d\overrightarrow{l} = -\int_S \frac{\partial \overrightarrow{B}}{\partial t} \cdot d\overrightarrow{S}. \tag{13}$$

Here, $\frac{\partial \overrightarrow{B}}{\partial t}$ represents the change in magnetic field with time. Equation 13 is also known as Faraday's law of electromagnetic induction in integral form. This form may be regarded as misleading since it describes two distinct phenomena: magnetic induction (due to a changing magnetic field) and electromotive force (due to movement of a charged particle through a magnetic field) [17]. Both phenomena cause the arising of an electromotive force, however solely magnetic induction produces an electric field. Another version of Faraday's law, the differential version, separates the two effects [17, 25]:

$$\nabla \times \overrightarrow{E} = -\frac{\partial \overrightarrow{B}}{\partial t} \cdot d\overrightarrow{S}. \tag{14}$$

Here, $\nabla\times$ represents the differential curl operator.

10.1.2 Electromagnetism and TMS

In case of TMS, electrical charge is stored in capacitors. This stored energy is periodically discharged from the capacitors through a conducting coil and produces a time-varying electrical field. In turn, this electrical field produces a transient magnetic field, causing a current to flow in a nearby conducting material, such as neurons. When of sufficient strength, this current may produce depolarisation of the conducting brain tissue located just under the coil [16]. The latter is illustrated in the following figure:

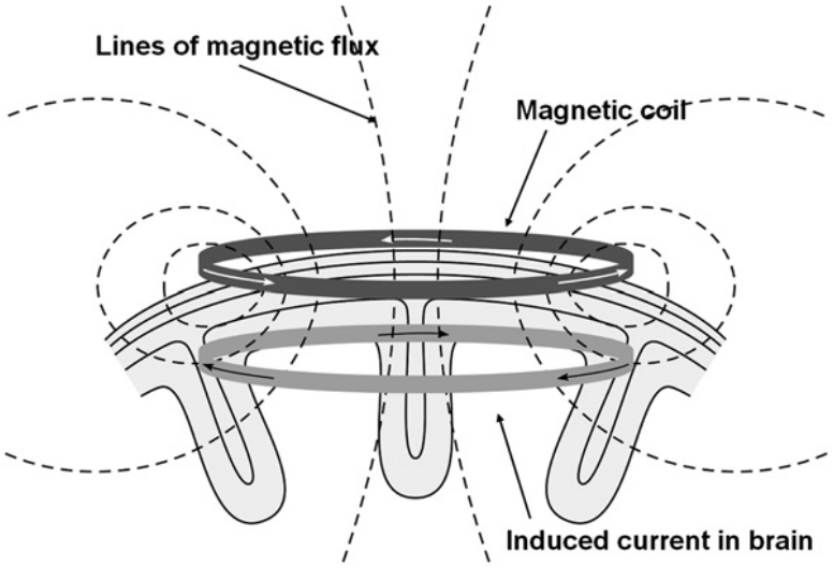

Figure 19: illustration of the current in the brain induced by TMS [24]

In magnetic stimulation, a brief, high intensity pulse is produced in a coil of wire. The magnetic field is produced perpendicular to the plane of the coil (placed tangential to the scalp). Perpendicular to the produced magnetic field, an electric field is induced. The voltage of the field itself may excite neurons, however likely more important for neuronal excitation are the induced current flows by the electric field [24].

Thus, an electrical field applied to neurons causes accumulation of induced current flows by the nerve membrane which may lead to excitation of these cells, resulting in driving of an intracranial electrical current in the brain by the electric field [17, 25, 33, 50]:

$$\overrightarrow{J} = \sigma \overrightarrow{E}. \tag{15}$$

Here, $\overrightarrow{J}$ is the current, and σ is the electrical conductivity of the brain [25].

10.1.3 Physiology

The flowing currents induced by TMS (see figure 19), may enter and exit the neurons. When currents remove charges from the membrane, a decrease in membrane potential will occur followed by depolarisation. The charge on the membrane capacitance increases when currents enter the cell membrane, leading to hyperpolarisation [70].
In case of sufficient membrane depolarisation, an action potential may be initiated. Whether an action potential is initiated depends on the spatial shape of the cell, the characteristics of the electric field, the coil geometry, and its position and orientation with respect to the nerve fibers [55, 70]. The magnitude and time-course of the gradient of the component (of the induced electric field) parallel to the nerve fibers has been shown to determine whether and where stimulation occurs [55]. The membrane current responsible for depolarisation or hyperpolarisation, is maximum where the spatial gradients of the axial current and the induced electric field are the largest. When applying a uniform field along an ideal cylindrical cell, membrane depolarisation or hyperpolarisation will not occur; no spatial gradient exists which is required for stimulation [55]. In reality however, uniform applied fields often stimulate neurons anyway. This is possible due to bends in the cell membrane: here, axial currents are non-zero and induction of membrane currents occurs, which may produce excitation or inhibition if sufficiently large [70].

The underlying mechanism of TMS is induction of an electric field in brain tissue for the purpose of neuronal excitation. In order to induce sufficiently strong motor cortex activation to lead to measurable MEPs, the cortical electric field should be in the order of 100 V/m. Cortical conductivity is $\sigma = 0.4$ m/s, thus an electric field strength on the order of 100 V/m results in a cortical current density of approximately 40 $\mu A/mm^2$ [25] (calculated through use of equation 15).

10.1.4 TMS pulse strength and energy duration

There are two factors on which the strength of TMS depends: the strength of the externally applied electric field and the temporal waveform of the stimulation pulse [25, 75]. The electrical pathway is formed by the capacitance and the resistance of the coil and behaves as a leaky integrator. Shorter stimulus pulses require less stimulus energy, however, decreasing the duration of the pulse does require an increase in stimulus amplitude. This principle is illustrated by figure 20 and the following differential equation:

$$\tau \frac{\partial V(t)}{\partial t} + V(t) = -\frac{dF(t)}{dt}. \tag{16}$$

Here, τ is the membrane time constant (100 to 200 μs), and $V(t)$ is the transmembrane potential measured from rest. $\frac{dF(t)}{dt}$ $\frac{\partial V(t)}{\partial t}$ is the change in force over time, and represents the change in transmembrane potential over time.

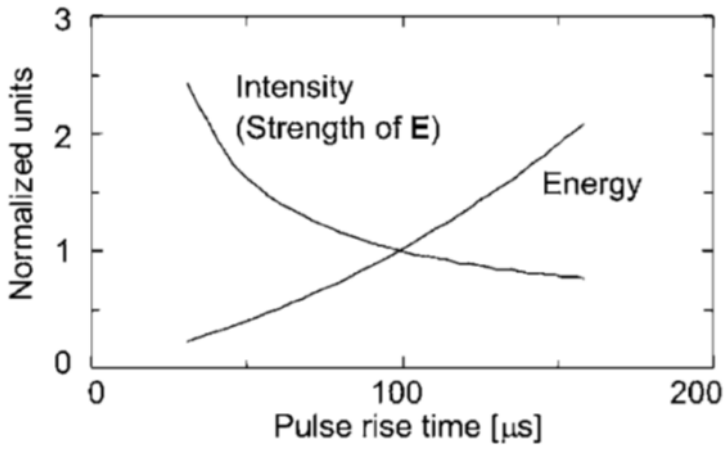

Figure 20: relationships between pulse strength-duration and energy-duration [25]
From this figure it can be seen how shorter stimulus pulses require less stimulus energy, however do demand an increase in stimulus amplitude.

Factors influencing the strength of the electric field induced in the brain by TMS are the shape of the coil, the location and orientation of the coil relative to the head, and the electrical conductivity structure of the head, scalp and brain [25].

10.1.5 TMS instrumentation

Typically, magnetic stimulators are used with coils of different sizes and shapes. Coils exist in different diameters and are butterfly (or figure-8) shaped or round. Round coils of a smaller size produce more focal stimuli, but with less intensity. An even more focal coil is the figure-8 coil and as the separate coils are wound with opposite current direction, the resulting current at the intersection of the 'wings' is increased [18, 25, 75].
For TMS brain studies, the figure-8 coil tends to be favoured because of its superior localization. However, for peripheral nerves it is difficult to find an orientation for the figure-8 coil to effectively couple with the target tissue. Therefore, circular coils are often used for peripheral nerve stimulation [18]. The biggest challenge for TMS is to reach a peak amplitude of up to 10,000 amperes with a very short (sub millisecond) pulse duration.

10.2 Appendix B

10.2.1 Complementing table

Table 3: Values for the mean amplitudes μ, standard deviations σ and calculated CoVs (through use of equation 6) used for the boxplots in figures 10 and 11

Participant	**Paradigm**	μ **90%**	μ **80%**	σ **90%**	σ **80%**	**CoV 90%**	**CoV 80%**
1	Standard	0.555556	0.53125	0.173916	0.115752	0.31305	0.217885
	Contralateral precontraction	1.261111	1.20625	0.458426	0.328764	0.36351	0.272551
	Tale	0.655556	0.65	0.111665	0.093541	0.170337	0.14391
	Double	5.558824	5.553333	0.734894	0.671185	0.132203	0.120862
	Triple	8.711765	8.833333	1.038598	0.713364	0.119218	0.080758
2	Standard	0.844444	0.75625	0.487878	0.244869	0.57775	0.323794
	Contralateral precontraction	0.355556	0.35	0.059835	0.05	0.168286	0.142857
	Tale	0.772222	0.76875	0.237593	0.208323	0.307675	0.270989
	Double	4.8	4.941667	2.103399	1.889646	0.438208	0.382391
	Triple	2.922222	2.90625	0.919272	0.752054	0.31458	0.258771
3	Standard	1.227778	1.0875	0.863974	0.432832	0.703689	0.398007
	Contralateral precontraction	0.85	0.80625	0.607591	0.496826	0.714813	0.616218
	Tale	1.222222	1.15	0.93782	0.835165	0.767307	0.72623
	Double	1.84	1.692308	2.051926	1.890243	1.115177	1.116962
	Triple	10.23529	10.36667	3.780125	3.537168	0.369323	0.341206
4	Standard	0.316667	0.31875	0.068718	0.063431	0.217006	0.198998
	Contralateral precontraction	0.282353	0.28	0.061695	0.05416	0.218502	0.193429
	Tale	0.633333	0.5875	0.384419	0.293417	0.606977	0.499434
	Double	0.455556	0.44375	0.149897	0.127322	0.329042	0.286923
	Triple	2.838889	2.8125	0.731922	0.577576	0.25782	0.20536
5	Standard	0.511111	0.475	0.368011	0.309233	0.720022	0.651017
	Contralateral precontraction	0.483333	0.48125	0.138444	0.128544	0.286435	0.267103
	Tale	0.455556	0.44375	0.138332	0.111629	0.303656	0.251557
	Double	0.522222	0.525	0.198762	0.182003	0.380607	0.346672
	Triple	1.411111	1.34375	0.470093	0.264501	0.333137	0.196838
6	Standard	0.533333	0.5125	0.197203	0.140868	0.369755	0.274864
	Contralateral precontraction	0.766667	0.75625	0.249444	0.209072	0.325362	0.276458
	Tale	1.25	1.2125	0.535672	0.444234	0.428538	0.366378
	Double	4.811111	4.75	1.287068	1.024085	0.26752	0.215597
	Triple	9.016667	9.025	2.215915	1.745888	0.245758	0.19345
7	Standard	1.544444	1.475	0.789671	0.613901	0.511298	0.416204
	Contralateral precontraction	0.833333	0.825	0.310913	0.277263	0.373095	0.336077
	Tale	0.972222	0.9625	0.315886	0.271282	0.324911	0.281851
	Double	2.227778	2.2125	1.102928	1.012963	0.49508	0.457836
	Triple	7.627778	7.75	1.825683	1.541104	0.239347	0.198852

Participant	**Paradigm**	μ **90%**	μ **80%**	σ **90%**	σ **80%**	**CoV 90%**	**CoV 80%**
8	Standard	0.638889	0.625	0.231274	0.207666	0.361994	0.332265
	Contralateral precontraction	1.316667	1.275	0.416667	0.258602	0.316456	0.202825
	Tale	2.583333	2.5625	1.080766	0.939332	0.418361	0.366568
	Double	3.127778	3.05	1.160845	0.878208	0.371141	0.287937
	Triple	8.222222	8.1875	0.748001	0.580813	0.090973	0.070939
9	Standard	0.358824	0.326667	0.344808	0.304339	0.960941	0.93165
	Contralateral precontraction	0.835294	0.82	0.436485	0.372738	0.522552	0.454558
	Tale	0.75	0.74375	0.214087	0.176666	0.28545	0.237534
	Double	3.522222	3.5625	1.236283	1.097654	0.350995	0.308113
	Triple	4.572222	4.55	1.682857	1.49541	0.368061	0.328661
10	Standard	0.311111	0.3125	0.109994	0.092702	0.353553	0.296648
	Contralateral precontraction	0.25	0.24375	0.111803	0.093332	0.447214	0.382902
	Tale	1.022222	0.99375	0.441658	0.376611	0.432057	0.37898
	Double	7.111111	7.1125	0.751952	0.714908	0.105743	0.100514
	Triple	6.822222	6.83125	0.881007	0.699749	0.129138	0.102433
11	Standard	0.111111	0.10625	0.031427	0.024206	0.282843	0.227823
	Contralateral precontraction	0.177778	0.16875	0.078567	0.058296	0.441942	0.345458
	Tale	0.261111	0.25625	0.100769	0.07881	0.385922	0.307549
	Double	0.171765	0.168	0.0884	0.086039	0.514657	0.512135
	Triple	0.916667	0.91875	0.318416	0.287704	0.347363	0.313147
12	Standard	1.266667	1.26875	0.454606	0.377026	0.3589	0.297163
	Contralateral precontraction	1.055556	0.99375	0.507961	0.378267	0.481227	0.380646
	Tale	1.1	1.06875	0.452155	0.388456	0.41105	0.363468
	Double	4.711111	4.55	2.93407	2.48998	0.622798	0.547248
	Triple	7.027778	7.025	1.668601	1.478809	0.237429	0.210507
13	Standard	0.582353	0.533333	0.332964	0.221108	0.571756	0.414578
	Contralateral precontraction	0.538889	0.53125	0.236029	0.205301	0.437993	0.386449
	Tale	0.933333	0.90625	0.433333	0.338136	0.464286	0.373116
	Double	1.944444	1.9625	0.539776	0.472857	0.277599	0.240946
	Triple	3.538889	3.5375	0.94642	0.816911	0.267434	0.230929
14	Standard	0.311111	0.29375	0.119977	0.07303	0.38564	0.248612
	Contralateral precontraction	0.272222	0.25625	0.159184	0.122315	0.584757	0.477326
	Tale	0.116667	0.1125	0.037268	0.033072	0.319438	0.293972
	Double	2.388235	2.32	2.32	2.988772	1.139708	1.251456
	Triple	4.205556	4.2	1.137967	0.990581	0.270587	0.235853
15	Standard	0.244444	0.23125	0.16131	0.140475	0.659903	0.607461
	Contralateral precontraction	0.788889	0.79375	0.319529	0.277193	0.405037	0.349219
	Tale	1.077778	1.0125	0.442914	0.28477	0.410951	0.281254
	Double	1.152941	1.14	0.358293	0.296198	0.310764	0.259823
	Triple	4.9	4.85625	1.287979	1.076434	0.262853	0.262853

10.3 Appendix C

10.3.1 BrainVision Analyzer steps

In order to effectively use a level trigger transformation, minor movements and noise need to be filtered out of the data.

Therefore, the function *IIR filters*, under *transformations* and *data filtering* is chosen. Next, the following screen appears:

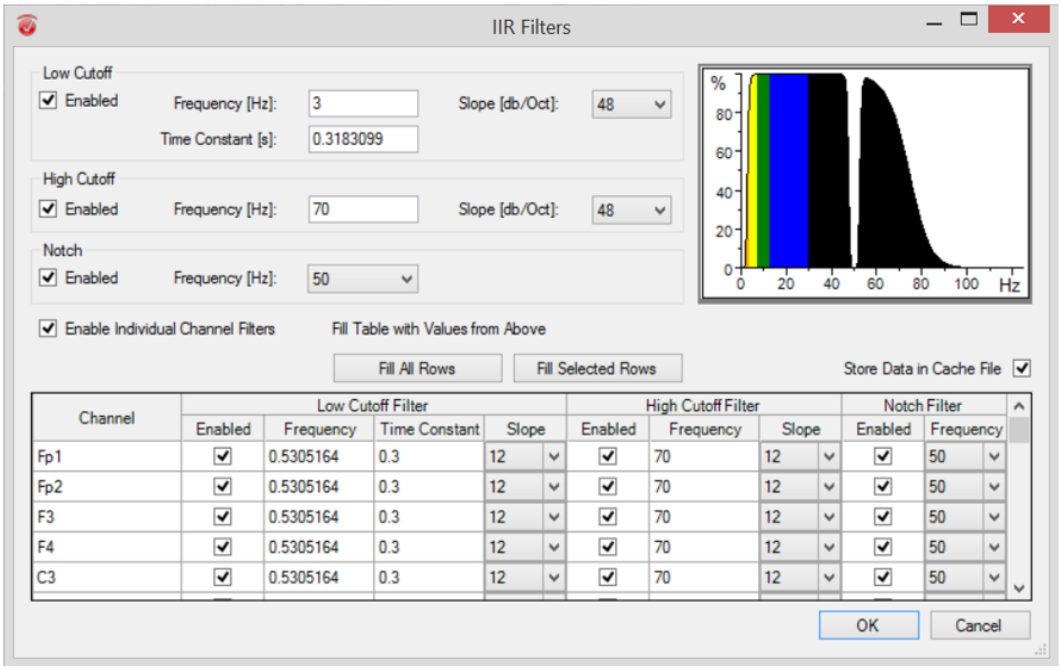

Figure 21: selecting transformations → data filtering → IIR filters will lead to the displayed options menu for setting filter characteristics

Filter settings for filtering during this stage of processing were as shown in figure 21.

For segmentation of the data in pre pulse segments in a later stadium of processing, first markers need to be placed on each pulse artefact. To do so, a *level trigger* is used which can be found under *transformations*. By selecting the *level trigger* function, the screen in figure 22 is displayed.

For the level trigger used during data processing of this study, settings were as shown in figure 22.

Now that markers have been placed, they have to be transported back into the raw unfiltered data. The first step to transport pulse markers back into raw data, is to export them out of the filtered data. Through selecting *markers* under *export*, the screen as displayed in figure 23 appears.

Here, only selected markers (namely TMSpulse markers) were exported out of the filtered data.

To import TMSpulse markers back into the raw data, first raw data is selected again for further processing. By selecting the function *import markers*, under *transformations* and *other*, the screen in figure 24 is displayed.

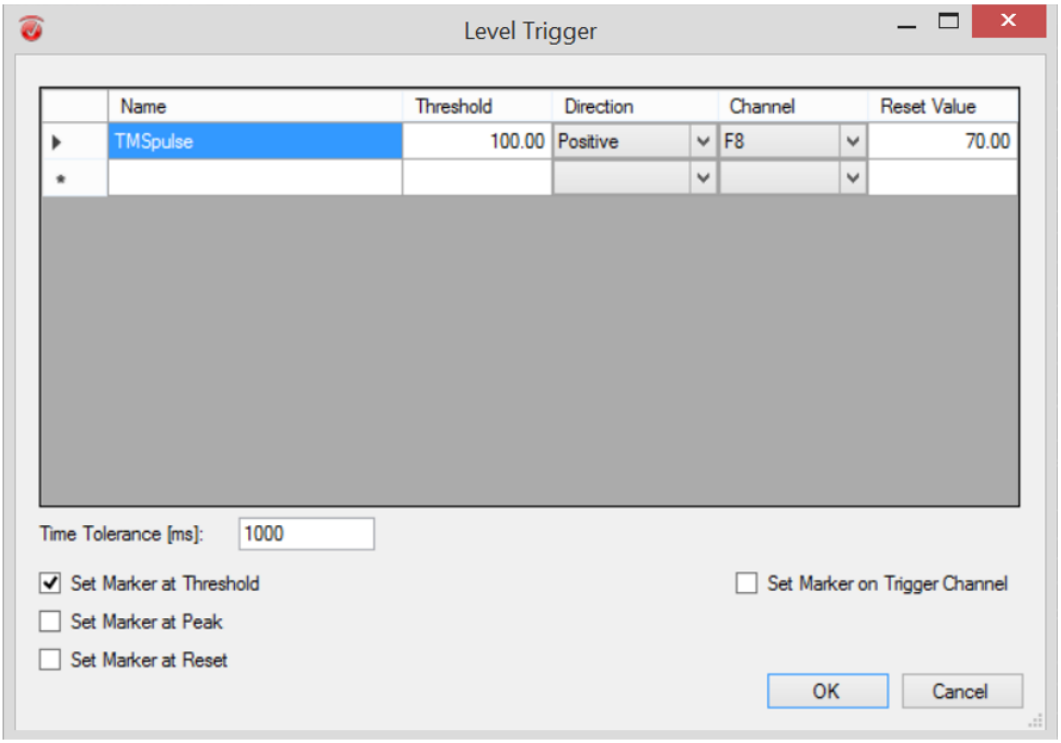

Figure 22: selecting transformations → level trigger will lead to the displayed options menu for setting the level trigger

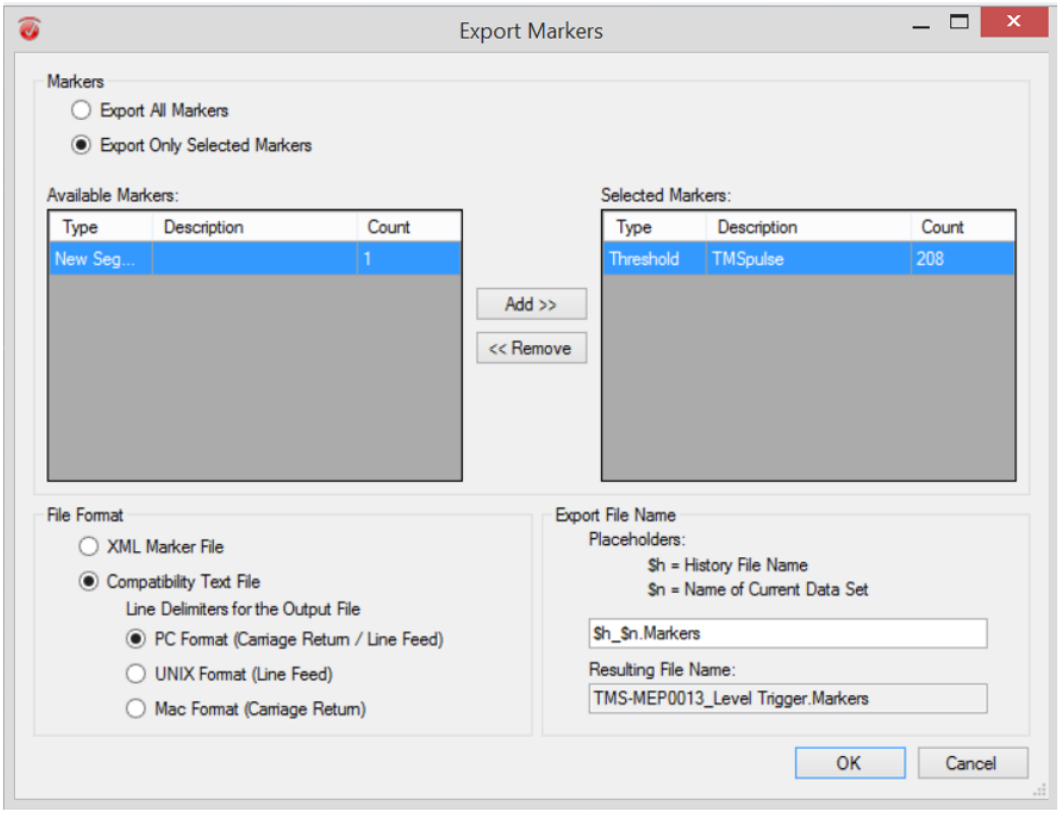

Figure 23: selecting export → markers will lead to the displayed option menu for exporting markers

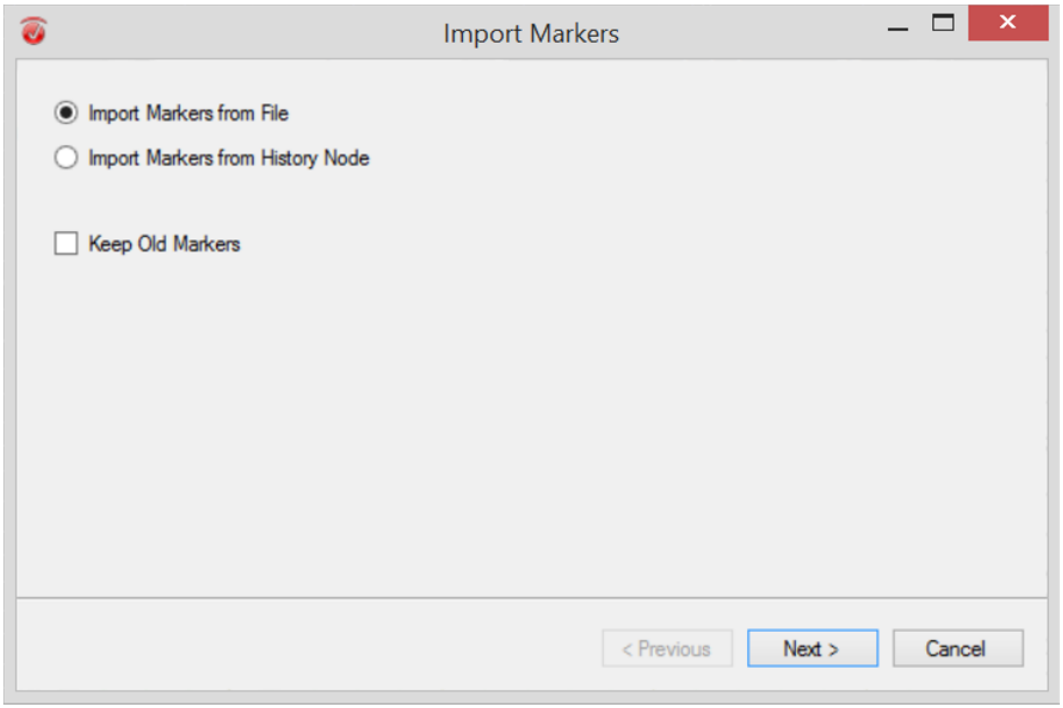

Figure 24: selecting transformations → other → import markers will lead to the displayed options menu for importing markers

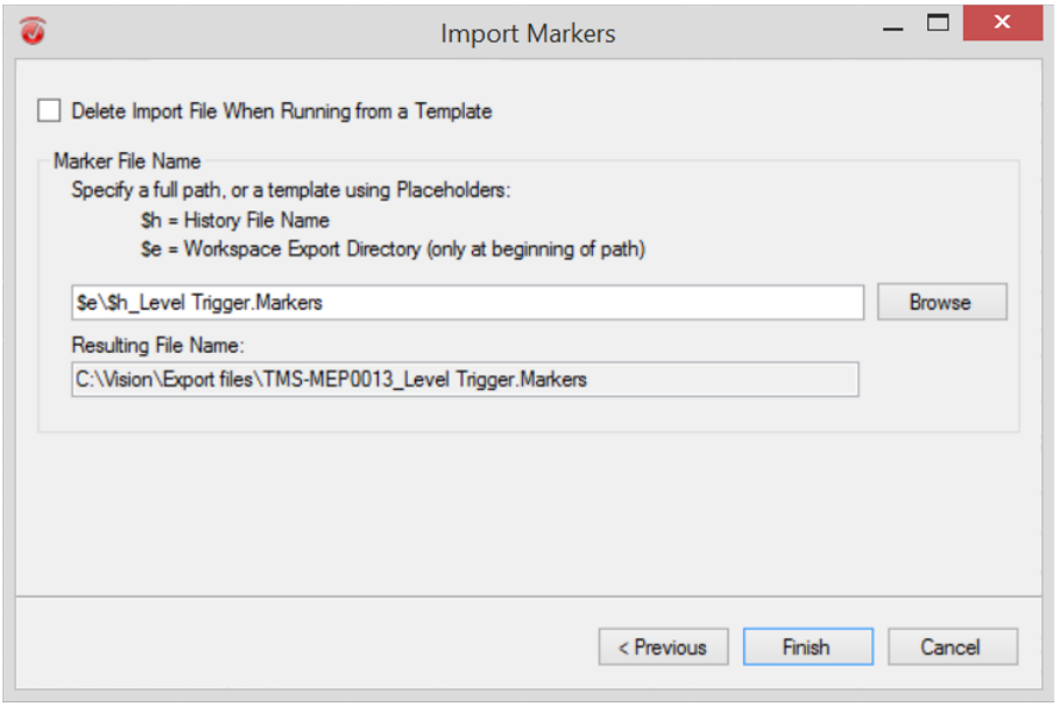

Figure 25: selecting import markers from file as shown in figure 24, will lead to the displayed options menu for importing markers

Through selecting import markers from file the previously exported markers can be selected for subsequent import. This step is shown in figure 25.
For filtering out slow movements and noise without discarding delta band information (1-4 Hz), data is filtered again by selecting *IIR filters* under *transformations* and *data filtering*. The screen that appears

is shown in figure 26. Please note that for this second time of filtering, low cut-off is set to 0.5 Hz.

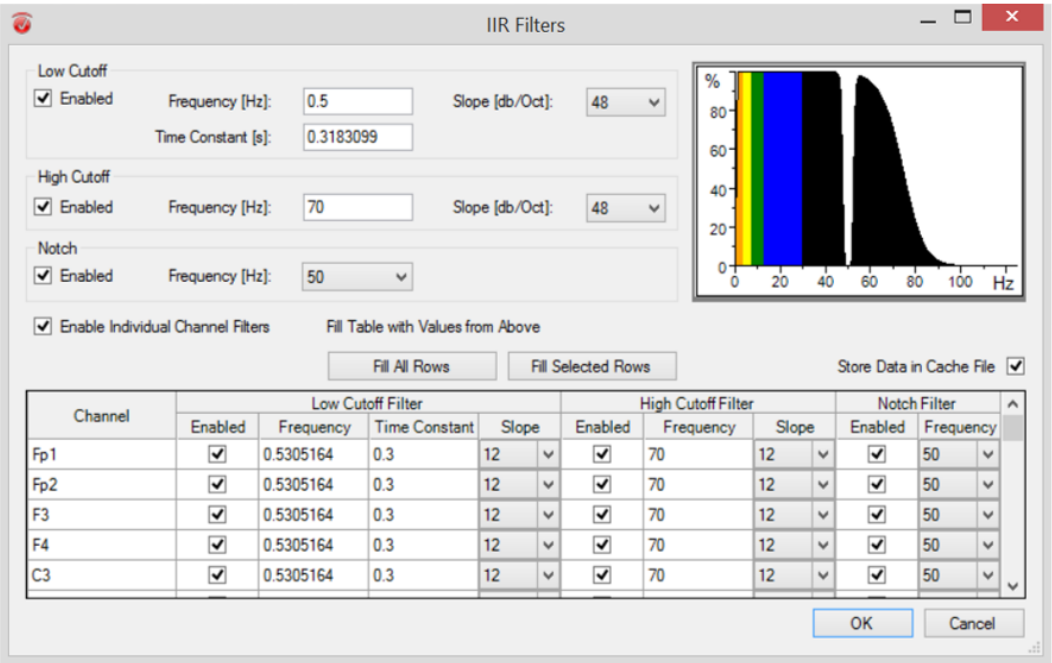

Figure 26: selecting transformations → data filtering → IIR filters will lead to the displayed options menu for setting filter characteristics. Please note the difference in low cut off value compared to filter setting in figure 21

For subdividing the data into the different tasks, subsequent segmentation based on data points is performed. Selecting *segmentation* under *transformations* will provide the screen displayed in figure 27. Here, the function *set new segments manually* is selected. By clicking next, figure 28 appears. Here, data points subdividing the data sets into the different tasks are manually entered.
The next screen that will appear in the segmentation sequence is figure 29 asking whether separate data sets for each segment should be created; here yes is selected. By clicking *finish*, this screen will close and five separate datasets are produced, each containing a single measurement task.

In order to subtract brain rhythm information 1400 ms prior to each pulse in a later stadium, segmentation based on marker position is performed next. Through clicking on the newly created segment 1, processing steps can be continued from here solely for segment 1 (the single pulse relaxed task).
Selecting *segmentation* under *transformations* will show the first screen of the segmentation wizard again (see figure 30). This time, the option *create new segments based on marker position* is chosen.

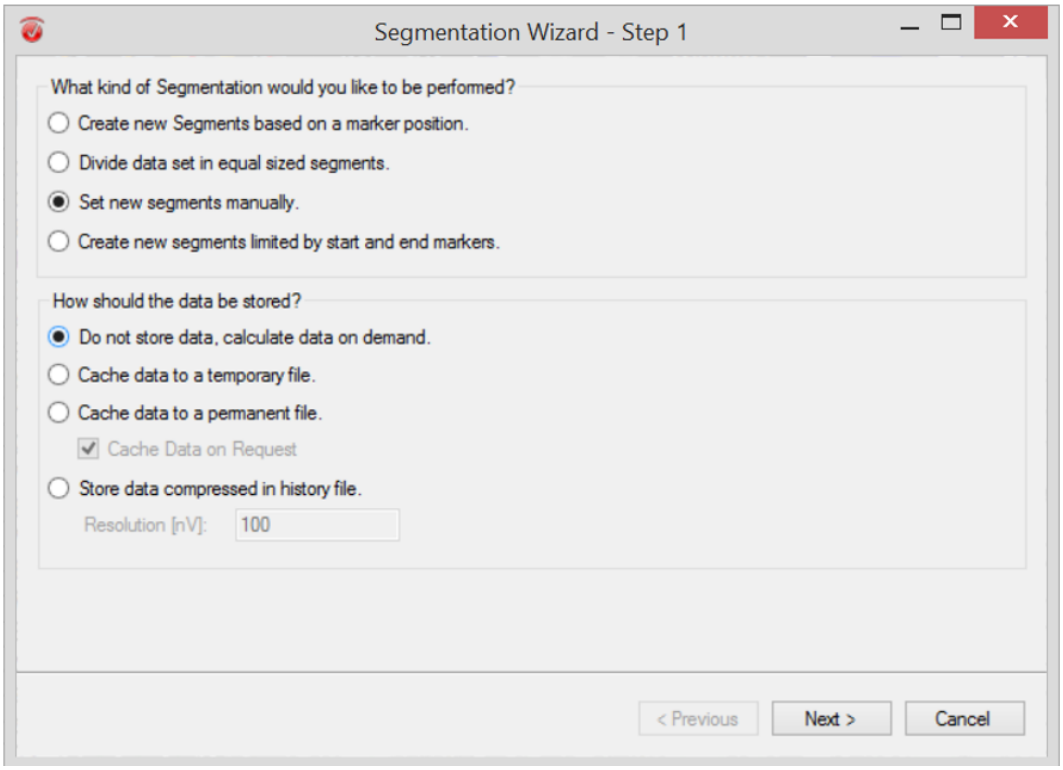

Figure 27: selecting transformations → segmentation will lead to the displayed options menu for data segmentation

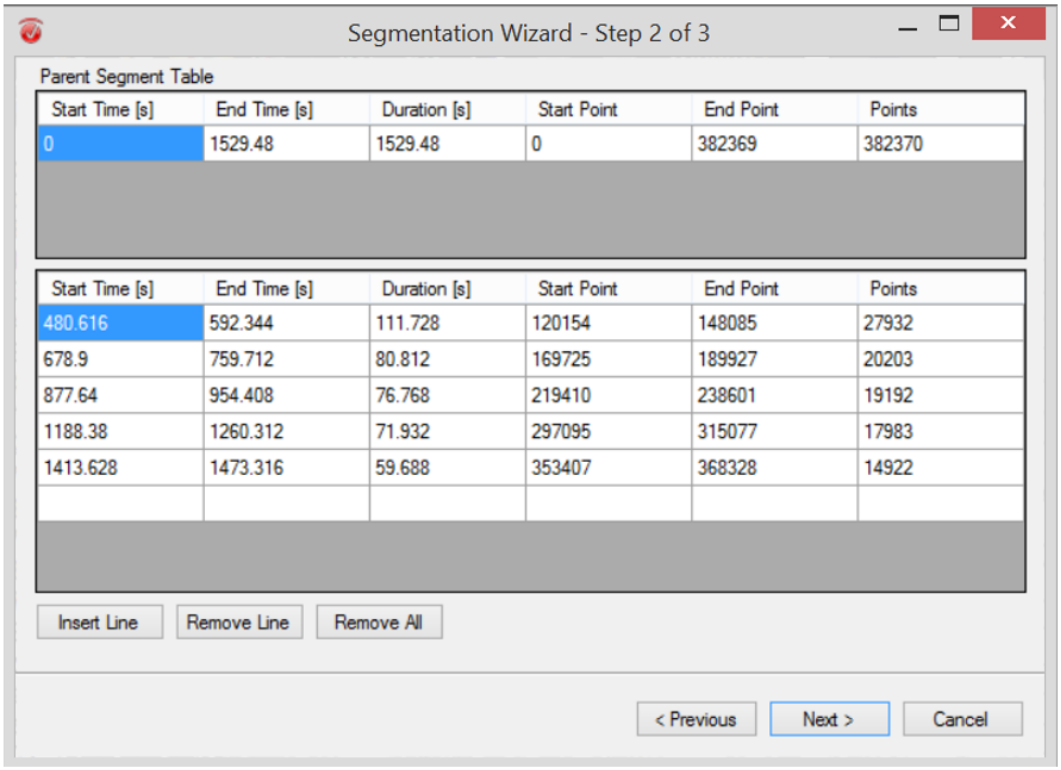

Figure 28: selecting set new segments manually will lead to the displayed screen for entering data points

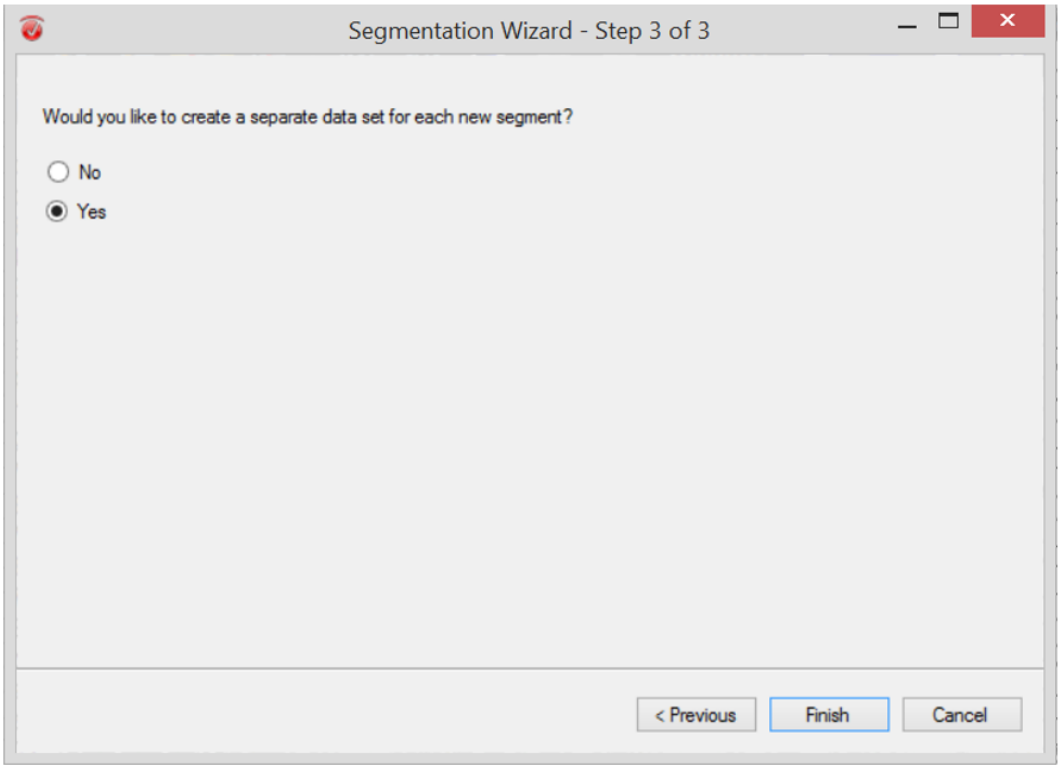

Figure 29: clicking next in the screen as displayed in figure 28 will lead to the displayed next options menu in the segmentation wizard. By finishing this step, five new datasets are produced, each containing a single measurement task

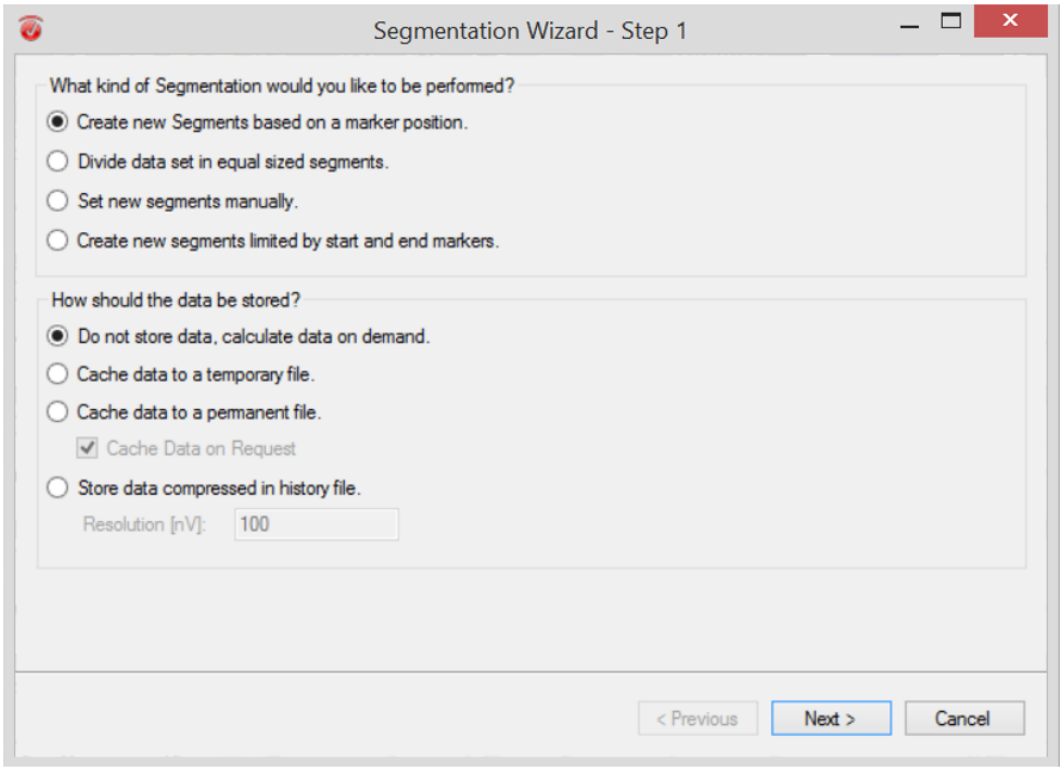

Figure 30: selecting transformations → segmentation will lead to the displayed options menu for data segmentation. Please note, in contrast to figure 27, here the option create new segments based on a marker position is chosen

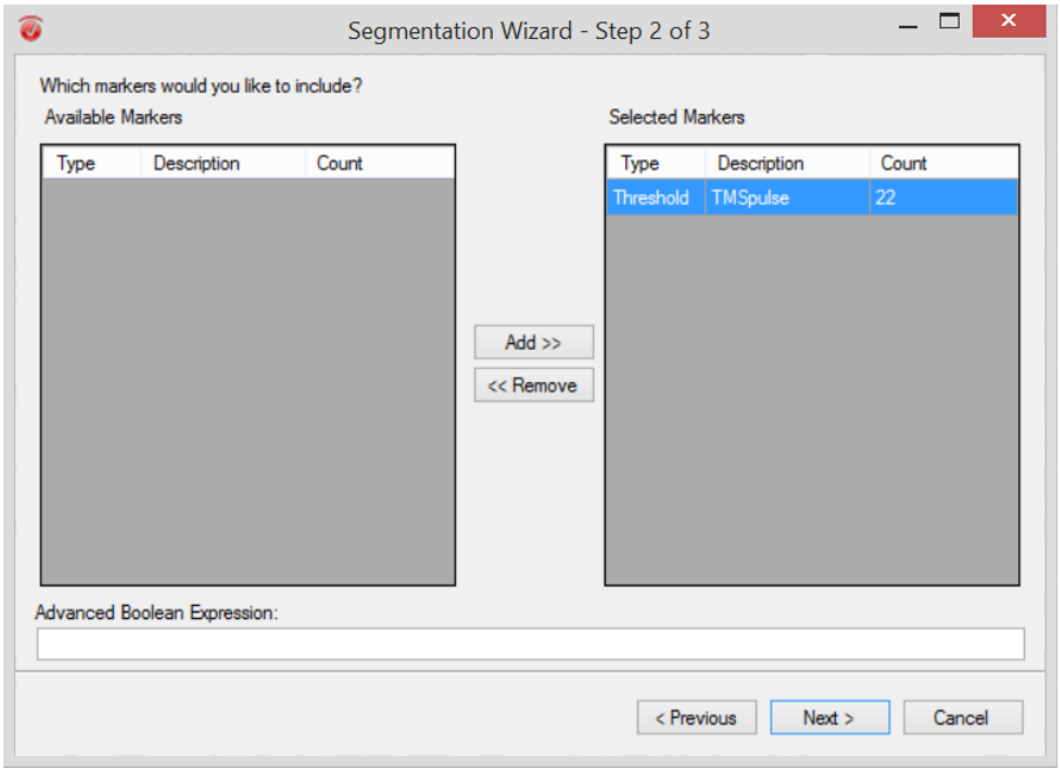

Figure 31: selecting next in figure 30 will lead to the displayed options menu for data segmentation based on marker position. Previously imported markers named TMSpulse are selected here

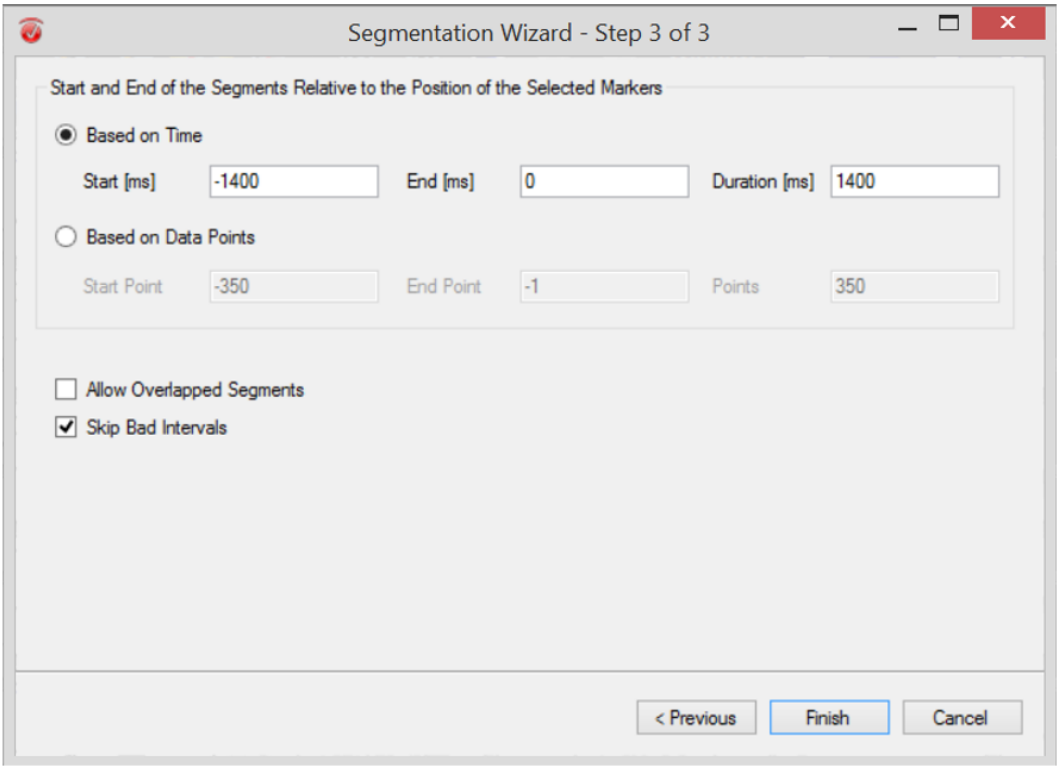

Figure 32: clicking next in figure 31 will lead to the displayed options menu for data segmentation based on marker position. Here, the period of 1400 ms prior to each pulse is selected for segmentation

By clicking *next* figure 31 will appear. Here, markers previously imported into the raw data (TMSpulse) can be selected for marker based segmentation. By completing this part of the segmentation, the menu in figure 32 will be displayed.
This options menu provides the tool for selecting a time period prior to each pulse/marker. A period of 1400 ms prior to each marker is specified and overlapped segments should not be allowed. By clicking finish, segment 1 (the normal task) will be segmented into pre pulse periods. This last step will be performed for every other segment (segment 2: contralateral contraction task; segment 3: tale reading task; segment 4: double pulse task; segment 5: triple pulse task).

To be able to extract frequency bands of interest, the next step is to transform the pre pulse segments per task segment to the frequency domain using Fourier transformation. Selecting *FFT* under *transformations* will provide the options menu in figure 33.

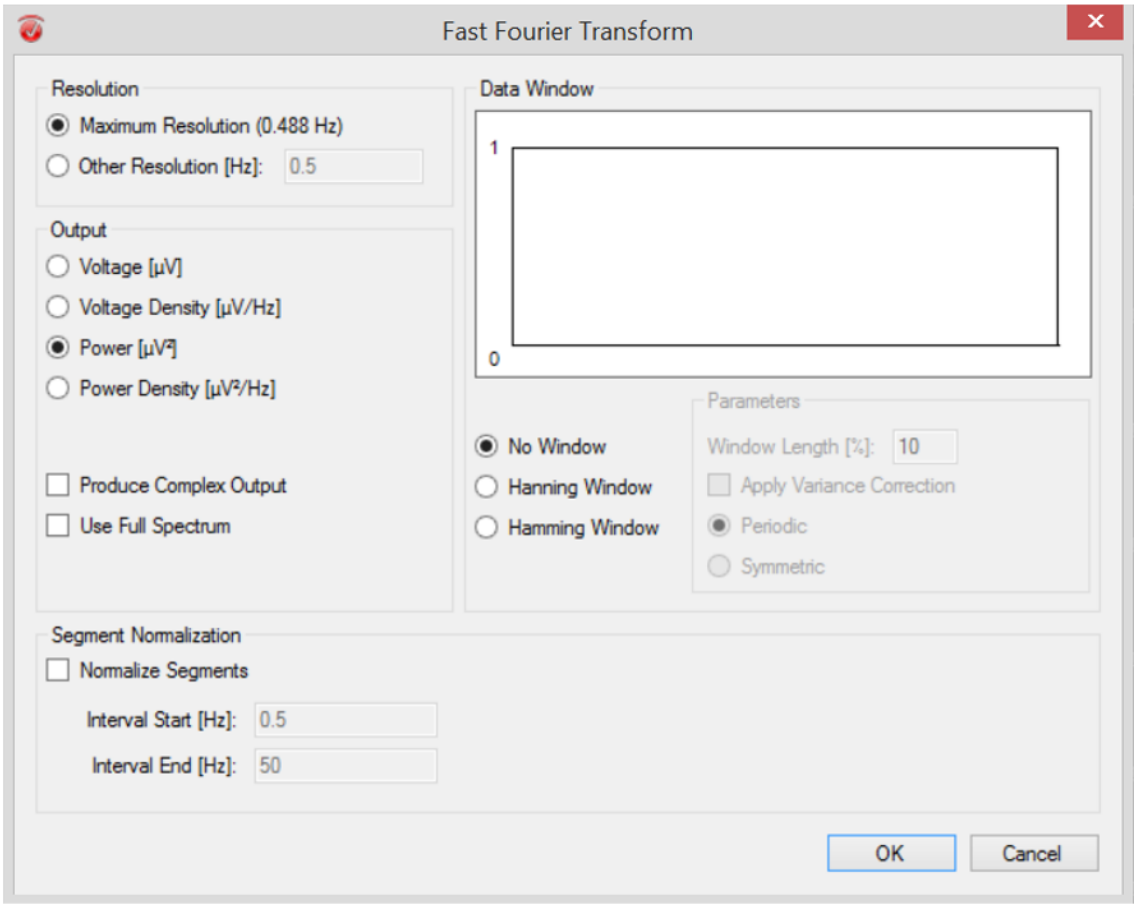

Figure 33: selecting transformations → FFT will lead to the displayed options menu for data transformation into the frequency domain

Here, settings as displayed in figure 33 are used.
The next step is to derive the relevant frequency band information from the data. For this purpose, the option *FFT band export* under *solutions* is selected. Figure 34 represents the screen that is displayed.

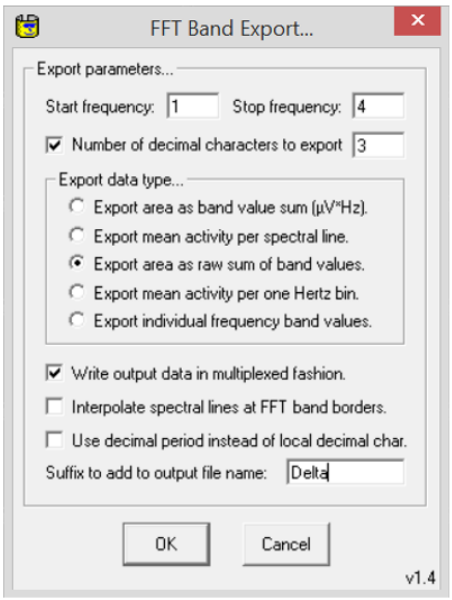

Figure 34: selecting solutions → FFT band export will lead to the displayed menu for FFT band information export

Here, the frequency band of interest is defined and exported for further analysis. This step is performed for each of the defined frequency bands of interest (delta: 1-4 Hz; theta1: 4-6 Hz; theta2: 6-8 Hz; alpha: 8-13 Hz; beta: 13-30 Hz) for each of the five task segments.

The latter step results in outcome as displayed in figure 35.

TMS-MEP0013_FFT5_FFTBandExport_delta - Kladblok

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	Fp1	Fp2	F3	F4	C3	C4	P3	P4	O1	O2	F7	F8
S01	7.046	3.823	13.048	1.761	4.241	4.077	2.341	2.405	3.658	3.737	4.489	5.447
S02	21.892	101.217	7.602	14.672	2.648	7.063	6.690	5.786	10.139	9.676	8.610	42.737
S03	79.113	9.341	1.149	3.857	1.933	1.139	8.004	10.314	13.155	15.214	12.257	13.800
S04	45.770	2.589	1.405	1.149	1.925	1.301	3.286	2.660	4.662	5.977	3.585	2.682
S05	12.215	3.197	1.330	0.605	0.845	0.521	2.388	0.983	3.014	3.936	4.260	5.117
S06	18.404	91.029	5.729	5.702	1.214	1.498	2.726	2.808	4.651	4.400	8.624	18.591
S07	17.072	106.081	8.416	12.051	1.739	5.239	7.554	6.687	10.837	11.829	14.032	34.314
S08	28.436	2.223	0.990	2.034	0.549	1.148	2.068	2.211	4.671	4.485	1.560	6.676
S09	8.819	4.354	1.623	1.723	0.870	1.983	2.967	2.131	5.916	5.550	5.676	6.985
S10	22.763	106.914	6.694	7.811	1.715	2.970	5.933	6.983	10.368	11.192	8.071	19.217
S11	16.658	1.903	1.088	1.462	0.504	1.931	1.231	2.036	2.415	3.623	2.527	5.154
S12	8.039	2.019	2.777	1.472	0.738	3.252	3.174	2.924	3.623	3.715	2.244	4.268
S13	18.394	116.928	4.677	10.100	3.374	1.860	6.182	4.862	8.292	8.146	6.556	28.293
S14	33.218	3.202	2.303	1.055	0.823	1.252	2.679	3.700	5.124	6.642	4.315	4.974
S15	27.125	1.671	1.423	1.611	0.994	0.861	1.824	1.080	3.463	2.936	4.017	2.953
S16	27.283	84.319	5.519	5.796	2.487	1.635	3.691	3.246	4.822	4.374	20.431	12.942
S17	28.264	2.057	3.560	1.215	0.537	0.928	1.115	1.488	2.203	2.168	4.773	3.298
S18	4.617	2.610	3.386	1.553	0.812	2.728	1.106	1.895	1.438	2.505	5.525	3.260
S19	7.201	2.638	4.396	1.816	0.575	3.413	2.097	3.792	3.962	4.522	5.168	7.485
S20	4.883	3.825	10.309	1.941	1.027	2.288	1.677	1.857	1.956	2.167	5.426	2.585

Figure 35: delta band area information per pre pulse period for a single task segment (all channels are included in this outcome, however cannot be shown due to limited space)

For each frequency band of interest per task, similar outcome files as displayed in figure 35 are obtained.

An overview of different steps performed in a single subject to analyse frequency bands of interest of a single tasks can be observed in the following figure.

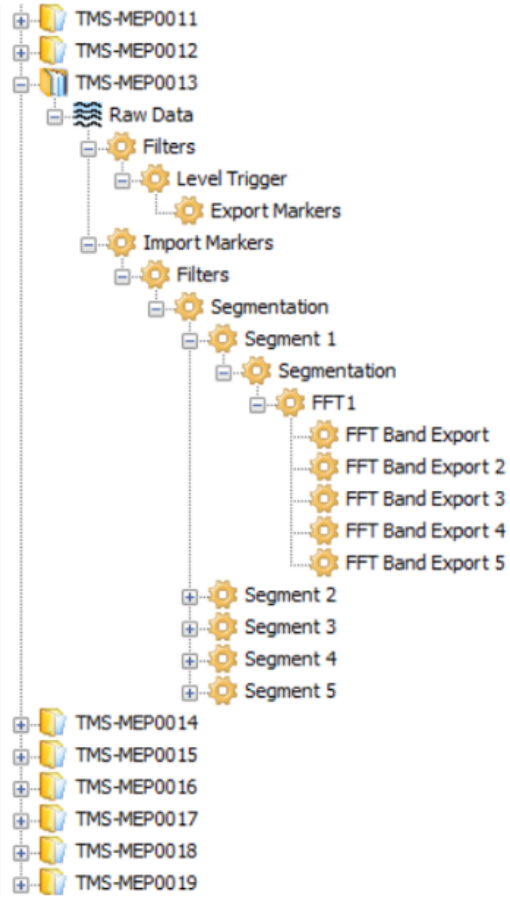

Figure 36: illustration of the template used in a single participant for a single task to export desired frequency band information

The processing steps as described above have been conducted for each of the 20 healthy participants for each of the five tasks.

10.4 Appendix D

10.4.1 Synergy steps

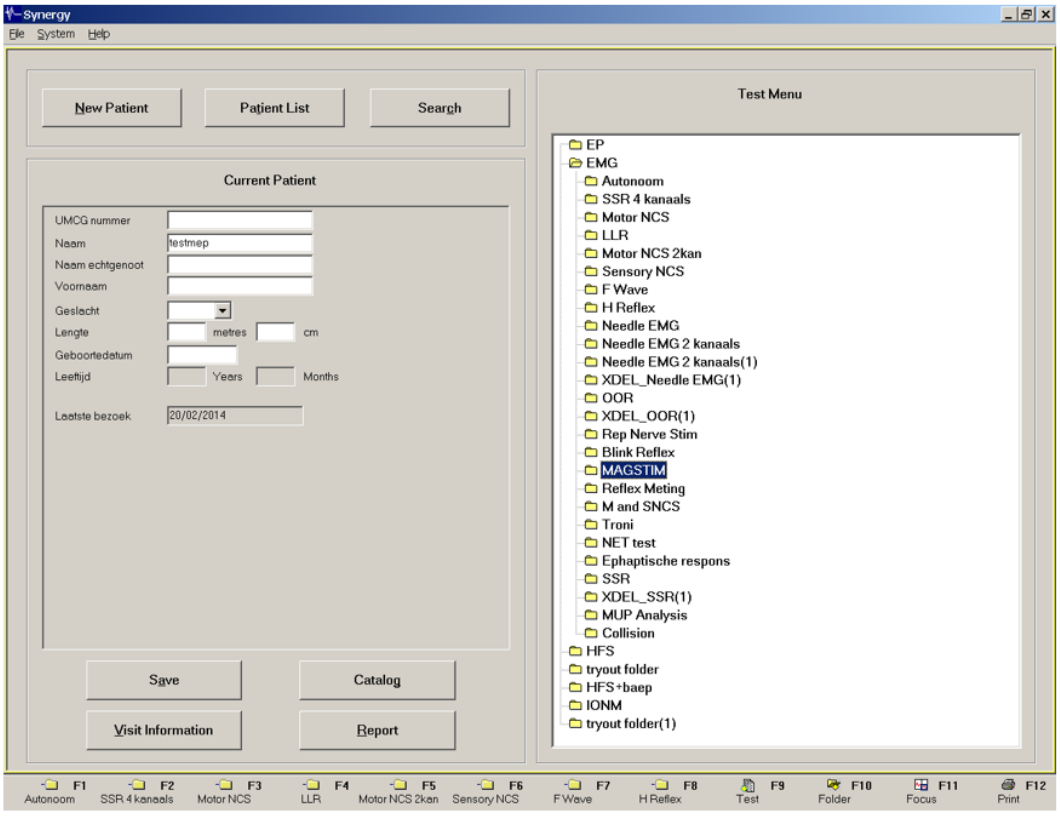

Figure 37: the startscreen of Synergy (Synergy NCS EMG EP IOM system, Optima Medical Ltd, Guildford, Surrey, UK)

For measurement of MEPs the *Magstim* option is selected under *EMG*.
Next, the screen as displayed in figure C2 will appear. Here, the measurement protocol *Medianus APB* for *both* sides is selected.

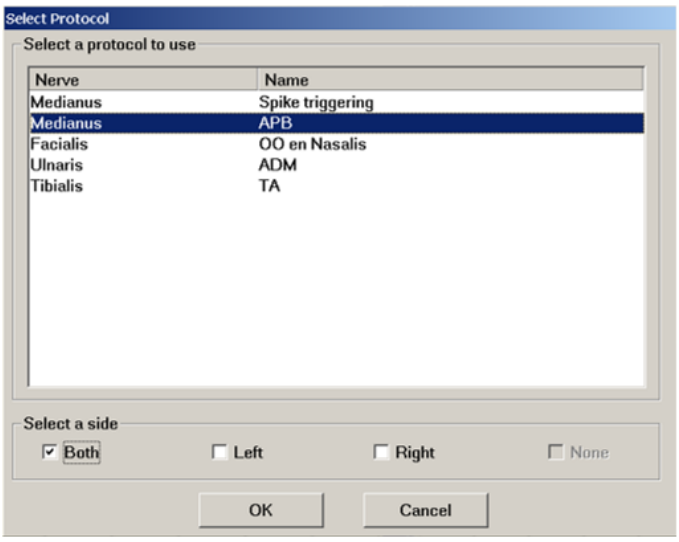

Figure 38: the screen for selecting the measurement protocol