Advances in detecting neuronal EEG signals during electromagnetic brain stimulation

Tuomas Mutanen
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The combination of transcranial electromagnetic brain stimulation (TEBS) and electroencephalography (EEG) enables direct modulation of neuronal activity at different cortical areas while simultaneously measuring the resulting response. Recording EEG concurrently with TEBS, however, is technically challenging. This Thesis attempts to solve several methodological problems related to combining EEG with the transcranial magnetic stimulation (TMS) and transcranial alternating current stimulation (tACS) techniques.

A major problem with TMS–EEG is that TMS easily activates cranial muscles, which results in large EEG disturbances that mask cortically evoked responses. This work demonstrates how to avoid muscle artifacts when recording the data, as well as how to suppress the remaining artifacts in the offline signal processing with minimal distortion in the signals of interest.

The common approach to study the cortical effects of TMS is to concentrate on the average evoked EEG response. This approach fails to inform us of how TMS affects the ongoing brain activity. This Thesis introduces quantitative tools to measure the TMS-induced changes in the brain-activity dynamics. The results indicate that TMS shifts the brain to an unnatural state, which is manifested as facilitated EEG variability.

TEBS–EEG signals often suffer from transient noise and artifact components that are present only in certain channels or trials. This Thesis shows how the disturbances can be automatically cleaned so that the collected data are optimally utilized and that the time used in signal processing is decreased. The presented approach is directly usable also with conventional EEG and, e.g., with magnetoencephalography data.

Combining EEG with concurrent tACS is difficult because of the large tACS-related EEG artifacts. The results presented in this Thesis indicate that the tACS-related EEG artifact can be successfully scaled down while preserving meaningful neuronal responses at the stimulation frequency.

This Thesis includes significant methodological advances that help to measure neuronal EEG signals in the harsh electromagnetic environment during TMS and TACS. The presented work enables a more flexible and reliable use of TEBS–EEG to study new brain areas and cognitive processes. The new tools help to utilize the gathered data fully, decreasing both the data acquisition and analysis times. Consequently, TEBS–EEG becomes a more feasible tool for novel research paradigms, as well as for clinical applications.

**Keywords** Transcranial magnetic stimulation, Transcranial alternating current stimulation, Electroencephalography, Artifact rejection, Noise cancellation

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Yhdistämällä transkraniaalinen sähkömagneettinen aivostimulaatio (TSAS) avosähkökäyrämittaussa (EEG) on samanaikaisesti mahdollista sekä suoraan säädellä että mitata eri aivokuorialueiden aktiivi suutta. EEG-mittaukset suorittaminen yhtäaikaisesti aivostimulaation kanssa on kuitenkin teknisesti haastavaa. Tämä väitöskirjatyö pyrkii ratkaisemaan useita ongelmia, jotka liittyvät transkraniaalisen magneetti- (TMS) ja vaihtovirtastimulaation (tACS) kanssa rinnakkain suoritettaviin EEG-mittauksiin.

Merkittävä ongelma TMS–EEG-mittauksissa on se, että magneettistimulaatio aktivoi herkästi pään alueen lihaksia. Tämä näkyy aivosähkökäyrissä suurina heilahteluina, jotka peittävät aivosähkökäyriltä peräisin olevat vastaukset. Väitöskirjatyön menetelmistä esitteellä, miten lihaskuviot voidaan välttää mittauksissa, sekä kuinka jäljelle jäävät lihashäiriöt voidaan poistaa myöhemmin jälkikäsittelyssä ilman, että tutkimuksen kannalta olot kärsivät.


TSAS–EEG-signaalit käsivät usein kiihdytävistä häiriöistä ja vääritymistä, jotka ovat näkyvissä vain tiettyissä kohtauksissa tai anturissa. Tämä työ näyttää, kuinka kohinaa on mahdollista poistaa tai vähentää, niin että kerätty aivosähkökäyrän mittaukset ovat realistisemmat ja lisäävät mittauksessa vastaavan erityisellä hermollisilla vastaissa mita saattaa käydä mahdollista.

Tämä väitöskirja sisältää useita merkittäviä menetelmäparannuksia, jotka auttavat mittaukseen suorittaa matalle erilaisaumoissa elävittäen erilaisia merkityksellisiä hermollisista vastaista, minkä mahdollistaa paremman mittaukseen suorittavaksi käytössä. Tämä Työ auttaa parantamaan ja määrätä aivosähkökäyrän mittaukseen suorittamaan mitalle erilaisaumoissa elävittäen erilaisia hermollisista vastaista, minkä mahdollistaa paremman mittaukseen suorittamaan mitalle erilaisaumoissa elävittäen erilaisia hermollisista vastaista.
First and foremost, I want to express my gratitude to my Thesis instructor and supervisor Prof. Risto Ilmoniemi. I could not have wished for a better mentor to lead me into the world of science. I feel privileged to have worked with such an inspiring and visionary researcher. Thank you for teaching me critical thinking and the importance of scientific integrity.

Committing oneself to scientific work would not be possible without the funding bodies. I want to thank the Finnish Cultural Foundation and the Foundation for Aalto University Science and Technology for supporting my Thesis financially.

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Research is always team work and I would like to acknowledge all of my collaborators who were involved in the included publications, Matleena Kukkonen, Sara Liljander, Dr. Toralf Neuling, Dr. Johannes Vosskuhl, and Prof. Christoph S. Herrmann. From my co-authors, I would especially like to thank Dr. Hanna Mäki for helping me to take my first steps in research and for being a great role model for a young research student, Dr. Jaakko Nieminen for his friendship, support, and invaluable feedback on my work, Dr. Matti Stenroos for constructive criticism and for building my understanding of bioelectromagnetism, Prof. Jukka Sarvas for teaching me a great deal of linear algebra and for entertaining me with his endless anecdotes, and finally, Dr. Johanna Metsomaa, for the delightful and fruitful collaboration and for sharing with me her exceptional mathematical insight.

While doing my PhD in the TMS group, I was able to work with some great people, Dr. Julio Hernandez-Pavon, Niko Mäkelä, Dr. Lari Koponen, Dr. Selja Vaalto, Sergei Tugin, Minna Pitkänen, Victor Hugo Souza, Aino Tervo, and Karita Salo. Thank you for all the endless discussions, pleasant collaboration, and fellowship. From the Department of Neuro-
Preface

science and Biomedical Engineering (NBE), I would also like to mention Prof. Lauri Parkkonen who was willing to share his expertise whenever requested, as well as the administrative staff: Eeva Lampinen, Mari Kaarni, Marita Stenman, and Henrika Wilkman, for helping in any practical matters.

I would like to thank the Biomag Laboratory for providing the equipment and facilities for conducting most of the experiments included in this Thesis. I would like to thank especially the head of the laboratory Dr. Jyrki Mäkelä and the lab engineer Dr. Juha Montonen for their support.

An important aspect of any workplace is the social atmosphere. In addition to the colleagues already mentioned, I would like to thank the rest of my friends at NBE, including Dr. Ilkka Nissilä, Dovile Kurmanaviciute, Joonas Iivanainen, Ivan Zubarev, Antti Rantala, Antti Mäkinen, Rasmus Zetter, Dr. Ville Mäntynen, Koos Zevenhoven, Teemu Turunen, Marja Pitkänen, Mikko Nyrhinen, Pauliina Hirvi, and many more for creating such a friendly and cozy environment.

In addition to my colleagues, I wish to mention my fellow Aalto students, Taneli Kari, Jere Mäkinen, Klaara Viisanen, Armi Tiihonen, Antti Vepsäläinen, Erkki Laurila, Sampo Hämäläinen, Dr. Samuli Autti, and many others, who have offered valuable peer support as well as countless priceless memories.

At the end, I would like to express my deepest appreciation to my family. Thank you, Mom, Dad, and Hanna, for your support in my studies and life in general. And my beloved wife Riina and my loyal companion Rudolf, thank you for being in my life and for taking my mind out of the work when I needed it. Thank you all for your love and care.

Glasgow, December 5, 2017,

Tuomas Mutanen
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List of Publications

This Thesis consists of an overview and the following Publications which are referred to in the text by their Roman numerals.


V Toralf Neuling, Tuomas P. Mutanen, Johannes Voskuhl, Risto J. Ilmoniemi, Christoph S. Herrmann. Signal-space projection suppresses the tACS artifact in EEG recordings. Submitted. 31 May 2017

* The first two authors contributed equally to these studies.
Author’s Contribution

Publication I: “The effect of stimulus parameters on TMS–EEG muscle artifacts”

The author analyzed the data and was the main responsible for performing the experiments. He designed the experiments in collaboration with the other authors and wrote the paper with the second author.

Publication II: “TMS-evoked changes in brain-state dynamics quantified by using EEG data”

The author developed and validated the quantitative tools presented in the article. He analyzed the data and was the principal writer of the article.

Publication III: “Recovering TMS-evoked EEG responses masked by muscle artifacts”

The author developed the combined SSP–SIR approach with the second and third authors. He ran the simulations, analyzed the data, and was the principal writer of the work. The author helped in measuring parts of the data.
Publication IV: “Automatic and robust noise suppression in EEG and MEG: The SOUND algorithm”

The author invented and formulated the SOUND algorithm, developed the methodology, and wrote the manuscript with the second author. The author measured and analyzed the TMS–EEG and MEG data, while the second author ran the simulation studies.

Publication V: “Signal-space projection suppresses the tACS artifact in EEG recordings”

The author built the phantom head and analyzed the phantom data. He also contributed to analyzing the human data and to writing the paper.
# List of Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>CT</td>
<td>Computed tomography</td>
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<td>EEG</td>
<td>Electroencephalography</td>
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<td>EMG</td>
<td>Electromyogram</td>
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<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<td>ICA</td>
<td>Independent component analysis</td>
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<td>M1</td>
<td>Primary motor cortex</td>
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<td>MEG</td>
<td>Magnetoencephalography</td>
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<td>MNE</td>
<td>Minimum-norm estimate</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MSE</td>
<td>Mean squared error</td>
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<td>MSS</td>
<td>Mean state shift</td>
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<td>NBS</td>
<td>Navigated brain stimulation</td>
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<td>nTMS</td>
<td>Navigated transcranial magnetic stimulation</td>
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<tr>
<td>PCA</td>
<td>Principal component analysis</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>SIR</td>
<td>Source-informed reconstruction</td>
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<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<td>SOUND</td>
<td>Source-estimate-utilizing noise-discarding algorithm</td>
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<td>SSP</td>
<td>Signal-space projection</td>
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<td>SV</td>
<td>State variance</td>
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<td>SVD</td>
<td>Singular-value decomposition</td>
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<td>Abbreviation</td>
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<tr>
<td>tACS</td>
<td>Transcranial alternating current stimulation</td>
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<td>tDCS</td>
<td>Transcranial direct current stimulation</td>
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<tr>
<td>TEBS</td>
<td>Transcranial electromagnetic brain stimulation</td>
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<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
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<tr>
<td>tRNS</td>
<td>Transcranial random noise stimulation</td>
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1. Introduction

The brain is one of the most fascinating physical systems. Its mechanisms explain, for instance, the fundamentals of our self-awareness [60], consciousness [231], memories [78], language [187, 188], and emotions [197]. It could be said that these cognitive capabilities define us as human beings [210]. Not surprisingly, this unique feature of the brain has inspired a vast number of brilliant scientists to commit their careers to understand the functioning of the nervous system.

Regardless of the broad scientific interest towards the different aspects of cognition and neuronal physiology, there are still many aspects in the brain functioning that are poorly understood [81, 92, 3]. Even today, the limiting factor in cutting-edge brain research is often the available technology and methodology [126]. We need more robust, precise, and accurate methods to quantify all the relevant phenomena in the brain at different time and spatial scales. Due to various technical and methodological challenges, a great deal of applied mathematics, signal processing, instrumentation, and physics work is still required before we can say that we understand how the brain works. This Thesis tackles technical issues related to a very specific brain imaging technique, i.e., combined transcranial electromagnetic brain stimulation (TEBS) [91, 171] and electroencephalography (EEG) [152]. The aim of the presented work is to develop recording and signals-analysis methodologies that help researchers to separate and quantify the neuronal signals of interest in the measured TEBS–EEG data.

Nowadays, utilizing the modern technology, it is possible to study certain aspects of the brain in fine detail. For instance, with the state-of-the-art magnetic resonance imaging (MRI) [24] devices it is possible to
gather elaborate information of the brain structure. We also have several recording and imaging approaches that can be used to answer very specific questions related to brain functioning. For example, when activating different parts of the human cortex with different sounds, visual imagery, or tactile sensations, we can localize the neuronal reactions to the given stimulus [76, 5, 184, 75] with good spatial and excellent temporal resolution.

Despite the significant advances in neuroscience, we still lack information of how brain processes, stores, and transmits information. One concrete challenge in answering these questions is that it can be difficult to design a measurement paradigm that allows to excite an arbitrary brain region or to modulate a certain cognitive process, while simultaneously measuring the resulting changes in the neural activity. In principle, the introduction of combined TEBS–EEG provided a flexible tool to perturb and measure the brain. In TEBS, the brain activity can be directly affected by targeting electric fields to head, and with concurrent EEG we can measure the evoked changes in the brain functioning.

EEG is based on measuring scalp potentials that result from weak electric signals in the brain. Therefore, it is not a big surprise that recording EEG, while simultaneously modulating of the brain activity with relatively strong electric fields, can be technically challenging. This Thesis addresses some of the most severe methodological problems related to combining online EEG recordings with two different brain stimulation techniques, transcranial alternating current and transcranial magnetic stimulation (tACS and TMS, respectively).

In the overview part of this Thesis, the fundamentals needed to understand the included publications are reviewed. Thus, the relevant brain structures and mechanisms, as well as the basics of the applied measurements techniques are discussed. In addition, the main theoretical and analytical concepts that were used in Publications I–V are defined. Finally, the main results and implications of the included publications are discussed.
2. Aims of the Study

The main goal of this Thesis was to develop methodology for reliable quantification of the immediate TEBS-induced cortical effects using simultaneous EEG. More specifically, this works addresses the following methodological problems with the corresponding aims.

When performing TMS, large muscle artifacts may appear in the EEG data masking the neuronal signals of interest. In Publication I, our aim was to find optimal stimulation parameters to minimize the muscle-artifact contamination in TMS–EEG signals.

It is common to study only the average EEG responses to TMS. This approach, however, fails to observe the changes in the ongoing cortical activity. The aim of Publication II was to develop EEG measures to quantify the immediate effects of single-pulse TMS on brain-state dynamics.

It is sometimes impossible to choose the stimulation parameters so that the TMS-evoked muscle artifacts can be avoided completely. In Publication III, we aimed for a signal-processing method that rejects these muscle artifacts but still retains the neural EEG signals of interest undistorted.

Especially during electromagnetic stimulation, EEG data often suffer from a number of uncorrelated noise and artifact sources. The aim of Publication IV was to develop an algorithm that automatically identifies and rejects uncorrelated measurement noise.

tACS–EEG data suffer from severe artifact signals due to the stimulating currents. In Publication V, our aim was to demonstrate with phantom recordings that the tACS-induced EEG artifact can be suppressed using appropriate filtering techniques.
Aims of the Study
In this chapter, we review the background of the combined TEBS–EEG methods. We first consider the anatomy and mechanisms of the brain that are essential, both for explaining the working principle of TMS and tACS, as well as for understanding the generation of the EEG signals. We then explain the basis of the recording and stimulation techniques applied in this Thesis. Finally, we discuss the technical and methodological challenges related to combining TEBS with simultaneous EEG recordings and comment briefly the previously suggested solutions to these problems.

3.1 Brain structures and mechanisms influenced by electromagnetic brain stimulation

The whole brain consists of about 100 billion neurons that are heavily interconnected [232]. Together, the complex network of the brain cells constitutes an organ that conducts our thoughts, memories, actions, and observations.

The brain can be divided into several subparts that have their own functions and structural properties. The major parts of the brain are the brain stem, diencephalon, cerebellum, and the cerebrum [232]. Most of the brain-stimulation and EEG literature study phenomena taking place in the cerebrum, which is the superior, large, folded structure. Cerebrum is covered and protected by the cerebrospinal fluid (CSF), the skull, and the scalp [232].

The most superficial layer of the cerebrum is called the cerebral cortex. The cortex is a 2–4 mm thick layer of gray matter. Gray matter holds billions of cortical neurons, which are organized into layers, each layer
having its specialized function [23, 232]. One of the most common cell types in the gray matter is the pyramidal cell, playing a key role in the cortical information processing [214]. Pyramidal cells are also essential in the context of this Thesis, as they are believed to be the essential elements in generating the measurable EEG signals [152]. Pyramidal cells are also thought to be the primary targets of the TEBS [141]. Another important cell type in the gray matter is the interneuron [250], which most likely has its own role in the TEBS-activation processes. Underneath the gray matter, is the white matter consisting mainly of nerve fiber tracks, which transmit information between different brain areas.

The signaling of neurons is based on the electrophysiology of the nerve membrane. Due to the ion concentration difference between the intra- and extracellular spaces, there is a constant negative voltage across the membrane, called the resting membrane potential [99]. The main concentration gradient, largely determining the resting membrane potential, is maintained by the active cellular structures, sodium–potassium pumps [99]. If the membrane voltage is depolarized enough from the resting potential, some voltage-gated sodium channels open. As a result, there will be a fairly complicated cascade, which results in an electric pulse, called action potential [15], which is transmitted via a cable-like structure, axon [232]. The branched endings of the axon are connected to several other neurons, allowing the electric signal to be passed on to the connected cells.

The information carried by the action potentials is passed on from one neuron to another via synaptic connections [214]. There are both electrical and chemical synapses, the latter being responsible for most of the information transmission between neurons, in the mammalian brain [64]. The synaptic connections can be either excitatory or inhibitory [99, 214]. When the action potential reaches an excitatory (chemical) synapse, neurotransmitters that cause depolarizing post-synaptic currents in the connected cell, are released to the cavity between the pre- and post-synaptic neurons. In the case of an inhibitory synapse, the situation is the opposite. The released neurotransmitters cause hyperpolarizing post-synaptic currents in the receiving neuron.

A neuron receives signals from other cells through branched conductive projections, called dendrites, which are connected to the inputting neurons with synapses [214]. The received signals are integrated in the cell
body; based on the net polarizing effect of the excitatory and inhibitory currents, the integrating neuron either launches or does not launch an action potential [99].

The basic principle of TEBS is based on generating intracranial electric fields that affect the brain activity [141]. These fields depolarize some and hyperpolarize other neuronal structures, depending on the geometry of the imposed electric field as well as the fine structure of the brain. If the magnitude of the electric field is strong enough $(30–100 \text{ V m}^{-1})$ [104, 253, 113], it can trigger action potentials directly. When the electric field is weaker, it only affects the probability for neurons to fire action potentials.

With EEG, we can quantify cortical reactions by measuring the surface potentials on the scalp resulting from the post-synaptic activity [152]. If the electromagnetic brain stimulation affects the firing of the action potentials sufficiently, we can measure the resulting cortical effects indirectly with concurrent EEG.

### 3.2 Transcranial magnetic stimulation (TMS)

TMS is a technique that can be used to artificially activate a desired location in the cortex. A brief and strong magnetic pulse is applied to the subject’s head, inducing inside the brain an electric field that is capable of eliciting action potentials in the target neurons [12]. The first reported attempt to activate human brain with changing magnetic fields took place in 1896 when d’Arsonval placed the head of the subject inside an induction coil [39]. Later, in 1910, Thomson [227] and in 1911, Dunlap, Magnusson and Stevens, and Swinton [47, 131, 219] continued to study the visual sense with primitive magnetic stimulation. TMS, as we know it today, was introduced in 1985 by Barker et al. [12], who demonstrated that with magnetic pulses it is possible to activate the primary motor cortex (M1) sufficiently to evoke measurable activity in the peripheral muscles.

#### 3.2.1 Physics of TMS

With electromagnetic brain stimulation, we mean the generation and/or modulation of neuronal activity by creating an electric field inside the brain. In TMS, this electric field is induced by first generating a time-
varying magnetic field. Thus, it is important to notice that although we talk about magnetic stimulation, the brain activity is still ultimately affected by an electric field.

The working principle of TMS [91] is as follows. We bring a conducting coil next to the subject’s head and drive a strong time-varying current through the windings of the coil. The Biot–Savart law describes the magnetic field \( \mathbf{B} \) at a location \( \mathbf{r} \) generated by the current \( I \) in a coil (with winding pattern described by \( C \)):

\[
\mathbf{B}(\mathbf{r},t) = \frac{\mu_0}{4\pi} I(t) \oint_C \frac{d\mathbf{l}(\mathbf{r}') \times (\mathbf{r} - \mathbf{r}')}{|\mathbf{r} - \mathbf{r}'|^3},
\]

where \( d\mathbf{l} \) is a differential wire element at \( \mathbf{r}' \), \( t \) stands for time, and \( \mu_0 \) is the permeability of free space. From Eq. (3.1), we can see two things. Firstly, the generated magnetic field circulates the coil windings, indicating that the spatial pattern of \( \mathbf{B} \) depends on the geometry of the chosen coil. Secondly, the magnitude of the magnetic field decreases rapidly as the distance from the coil increases.

As the current \( I(t) \) varies in time, so does the generated magnetic field. Faraday’s law of induction states that the magnetic field induces an electric field \( \mathbf{E}_1 \) according to

\[
\nabla \times \mathbf{E}_1(\mathbf{r},t) = -\frac{\partial \mathbf{B}(t)}{\partial t}.
\]

Hence, for inducing an electric field it is essential that the applied magnetic field changes in time. Usually in TMS, the amplitude of the magnetic field is brought to as high as 1–2 T in some 100\( \mu \)s, which is enough to induce an electric field of approximately \( 100 \text{ V m}^{-1} \) in the cortex [91].

The consequence of the Biot–Savart and Faraday’s laws on the TMS-induced primary electric field is illustrated in Fig. 3.1. The direction of the induced primary electric field is opposite to the direction of the change of current in the TMS coil. The magnitude of the induced electric field is maximal close to the coil windings and decreases rapidly with the distance from the coil [244]. Because the chosen winding pattern determines the geometry of the magnetic field, it also defines the shape of \( \mathbf{E}_1 \).

The net electric field \( \mathbf{E} \) caused by TMS is a sum of the TMS-induced primary electric field \( \mathbf{E}_1 \) and the secondary field \( \mathbf{E}_2 \), which reflects the inhomogeneous conductivity profile of the targeted tissues [91]. The net TMS-induced electric field causes currents to flow in the conductive medium.
Background

Figure 3.1. A: A time-varying current in the TMS coil generates a magnetic field. The magnetic field passes freely through the skull and scalp. Inside the brain, the magnetic field induces an electric field. Adapted by permission from Macmillan Publishers Ltd: [71]. B: The magnitude of the induced electric field is in its maximum close to the coil windings and it declines rapidly as the distance from the coil increases. In a spherically symmetric case, possible conductivity discontinuities can be neglected and the net TMS-induced electric field equals $E_1$.

If the conductivity is not uniform along the preferred current paths, the charge is distributed unevenly, which results in a secondary electric field, $E_2$. Thus, $E_2$ depends on the net electric field and the conductivity profile of the tissue. The non-uniform conductivity profile of the intracranial space can have significant effects on the net TMS-elicited electric field [226].

### 3.2.2 Cortical effects of TMS

The effects of TMS on the cortical activity depend on two major factors, *i.e.*, the overall geometry of the TMS-induced electric field $E$ and how this
field couples with neurons in the microscopic scale.

Because the magnitude of the TMS-induced electric field decreases rapidly as a function of the distance from the coil, mainly the most superficial layers of the cortex are affected [253]. It is possible to increase the stimulation intensity so that relatively deep structures are also affected but this will consequently increase the induced electric field even more in the superficial structures. Thus, with TMS, it is not possible to exclusively stimulate deep brain structures [81]. When using figure-of-eight coils, the electric field is relatively focal also along the cortical surface; typically, the half-of-the-maximum electric field is reached on an approximately 3-cm × 5-cm area [154].

As mentioned in the previous section, the total induced electric field depends, not only on the coil shape and its distance to brain, but also on the conductivity profile of the head. On a macroscopic scale, the intracranial volume consists of at least three major compartments: the gray and white matters and CSF, each having its unique conductivity value [242]. Furthermore, the geometry of the cortex is very complex, consisting of folded and curvy structures. The complicated combination of different tissues results in a considerably inhomogeneous conductivity profile, which causes charges to accumulate at the conductivity discontinuities during the TMS pulse.

Simulations have shown that the structure of the cortex has two major effects on the net TMS-induced electric field [226]. The electric field is more focal compared to simple spherical head with homogenous conductivity inside the skull. Furthermore, the conductivity profile seems to cause directional selectivity; TMS induces a more pronounced electric field to those gyral structures that are roughly perpendicular to the primary electric field $E_1$. The effects of the gyral geometry on $E$ is illustrated in Fig. 3.2. The effect can be explained by the greater conductivity of CSF compared to gray matter. As the charges prefer to travel through the well-conducting CSF, there is an increased $E$ on the top of the gyri when $E_1$ is perpendicular to the gyrus.

To describe how TMS generates/modulates brain activity, it is necessary to understand how the net induced electric field affects the cortex on the cellular level. Experimental results indicate that the membrane of an axon close to the axon hillock is activated more likely than the mem-
Figure 3.2. The effect of the gyral geometry on the locality and shape of the induced electric field. A: The simulated TMS-induced electric field when taking into account the different conductivities of gray and white matters, as well as CSF. The arrows and the degrees indicate the orientation of the TMS coil with respect to the tangent of the gyrus. Those structures that are approximately perpendicular with respect to $E_1$ experience the strongest total electric field. B: The simulated TMS induced electric field when assuming a constant conductivity within the skull. Reprinted from [226], with permission from Elsevier.

brane at the cell body [11, 202, 9, 127]. The passive electric properties of a stimulated nerve fiber can be effectively explained with the cable model [200, 14]. According to the cable model, the most likely site of stimulation is at the bends of the axons [1]. Such bends can be found, for example, in the motor cortex in the axons that project from the walls of the central sulcus [161].

The exact orientation of the stimulus direction has a significant effect on the resulting cortical activity [189]. To maximize the effectiveness of TMS it is recommended that $E_1$ would be perpendicular with respect to the local gyrus [205]. The importance of the stimulation orientation might be explained by the macroscopic distribution of the net electric field (Fig. 3.2), the combined effects of the axonal geometry and the overall distribution of the pyramidal axons in the cortical surface, or a combination of these two effects.

It is not perfectly known which neurons are primarily activated by TMS. Some empirical results suggest that the corticospinal pyramidal cell is directly activated only by a sufficiently strong TMS pulse, and at weaker
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intensities, the excitation would be generated mostly indirectly via interneurons located in the more superficial parts of the gray matter [46].

3.2.3 TMS instrumentation

A TMS device consists of a TMS coil, which is connected to a large capacitor via an electric switch. The capacitor (C) is first charged to a few kilovolts. The charge is then passed in a few hundred microseconds through the coil. Because the coil windings and the cables have some resistance (R), and the coil has inductance (L), the stimulator electronics correspond to an RCL circuit [91].

The conventional approach to control the pulse is to use thyristors as a switch [91, 141]. The most common pulse waveforms with such devices are the monophasic and biphasic semi-sinusoidal waveforms. The biphasic pulses are evidently more effective in activating the cortex [128, 104, 213]. This is at least partially due to the passive membrane properties of the stimulated neurons; the membrane behaves like a leaky integrator [10], which facilitates the depolarization phase following the initial hyperpolarizing phase. It is also possible that biphasic pulses activate additional neuronal structures compared to monophasic stimulation [104, 213]. Recently, thyristors have been successfully replaced by insulated-gate bipolar transistors [176, 177, 175, 117], which allow much more flexible pulse forms.

As mentioned, the geometry of the TMS coil strongly affects the distribution of the induced electric field. The traditional TMS coil designs include circular [12] and figure-of-eight [34, 233] shapes, the latter being nowadays most commonly used. Compared to circular models, which produce a relatively distributed electric field that penetrates deeper into the cortex, figure-of-eight coils offer enhanced focality [244] (See Fig. 3.1). The recent advances [116, 207, 117] in the winding-optimization methods enable new coil designs that can have, for example, better efficiency or enhanced focality compared to the conventional solutions.

The aiming of TMS became significantly more accurate [102] when the navigated TMS (nTMS) was introduced [87, 205]. For the purpose of nTMS, the anatomical images of the subject are taken prior to stimulation. When delivering TMS, both the subject and the TMS coil wear
infrared-light reflecting markers that help the monitoring camera system to detect the relative movement of the coil with respect to the head of the subject. Navigation helps considerably in aiming TMS correctly to the anatomical structures of interest, as well as keeping the coil stationary with respect to the stimulation target.

3.2.4 Applications of TMS

One of the most well-established clinical application for TMS is the pre-surgical mapping [179, 222, 62]. TMS is especially practical in assessing the risks of a brain tumor surgery to the vital movement-representation areas. In practice, different points in M1 are activated with TMS while simultaneously measuring the resulting responses in the peripheral muscles. There have also been attempts to use TMS to map the cortical areas responsible for speech [125, 221, 84] but this has proven out to be challenging [178]. TMS significantly helps in planning the operation, as the pre-surgical mapping can be performed prior to opening the skull.

TMS has been successfully used also in depression treatment. Patients who are resistant to other anti-depression treatments can benefit considerably from the repetitive TMS that is delivered with pulse-train frequencies of 10–18 Hz to the left dorsolateral prefrontal cortex [166, 67, 173].

A third promising clinical application for TMS is the diagnosis and treatment of stroke patients [211]. The TMS-evoked muscle responses in the peripheral muscles in the upper limb may reflect the potential to recover from stroke [191, 48, 37, 217]. Repetitive TMS can be used for stroke rehabilitation. Usually this involves either high-frequency stimulation to the affected hemisphere [106, 220] or low-frequency stimulation to the contralateral hemisphere [61]. However, there is a large variability in the individual responses to this treatment [129].

In basic research, TMS has been used extensively to study M1 [169, 50, 255, 170], since the recording of the peripheral muscle activity with electromyogram (EMG) provides a practical readout of the TMS-evoked cortical activity in M1. TMS–EMG has been successfully used to study local intracortical inhibition and facilitation [33, 120, 256, 90], as well as interhemispheric inhibition [53, 42, 41]. TMS has been also utilized in various behavioral studies, e.g., for mapping the roles of different corti-
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cal regions in blind participants reading Braille letters [35], for assessing the importance of visual cortex in a visual imagery task [119], or for validating the significance of left and right supramarginal gyri in detecting and categorizing spoken sounds [77]. A disruption in the performance, when delivering TMS to a brain region of interest (ROI), has provided evidence about the causal relationship between the stimulated location and the studied cognitive function [168, 65].

By combining TMS with concurrent EEG, it is possible to record directly from the cortex how the TMS evoked activity is generated and spreading in the cerebrum. Currently, one of the most promising clinical applications of TMS–EEG is the measuring the degree of consciousness in patients suffering from unresponsive wakefulness syndrome [199, 190, 209]. TMS–EEG has also been proposed for the diagnosis of epilepsy [235, 107].

TMS–EEG has been utilized in several basic research projects. For example, the natural rhythms in the corticothalamic circuits [198], GABAergic neurotransmission [186], the role of connectivity in consciousness [138], and long-term potentiation [49] have all been studied with TMS–EEG. TMS–EEG has also proven to be a useful tool in studying the cortical reactivity in different conditions. For instance, it has been shown that alcohol reduces cortical excitability in the prefrontal lobe [103], whereas sleep deprivation increases cortical excitability in the supplementary motor cortex [89].

TMS–EEG has been considered an especially promising tool for probing the effective connectivity in the cortex [95, 114, 138, 115, 54, 56, 21]. A cortical location of interest is activated in a controlled way using TMS, while with concurrent EEG we can measure the spreading of the evoked activity to other cortical areas. In practice, however, studying connectivity with TMS–EEG be challenging for several technical reasons.

A major advantage of combining TMS with EEG compared to other TMS-compatible imaging modalities, such as functional MRI (fMRI) or positron emission tomography, is the superb temporal resolution. Furthermore, with EEG, we can observe more directly the TMS-induced effects on the neuronal activity. On the other hand, for instance fMRI provides a concurrent readout of the TMS-elicited activity with excellent spatial resolution. Indeed, there are number of TMS–fMRI studies, probing the connectivity in the human brain, e.g., [204, 203, 19, 122].
3.3 Transcranial alternating current stimulation (tACS)

Transcranial electrical stimulation is another widely used noninvasive technique to modulate the natural brain activity [171, 172]. Transcranial electrical stimulation can be divided into three different modalities: transcranial direct current stimulation (tDCS), transcranial random noise stimulation (tRNS), and transcranial alternating current stimulation (tACS), depending on the applied current waveform. In this Thesis, tACS and its application with simultaneous EEG is elaborated.

3.3.1 Physics of tACS

The main principle of tACS is straightforward. Typically, two electrodes are fixed on the scalp and a relatively weak current, of approximately 1 mA, is applied across these electrodes [251]. Consequently, the current must pass through the head. If the frequency of the applied current is sufficiently low, the quasistatic approximation [181] holds [142] and the tACS-caused electric potential $V$ can be written in terms of the Laplace equation:

$$\nabla \cdot (\sigma(r) \nabla V(r)) = 0,$$

(3.3)

where $\sigma(r)$ is the conductivity of the tissue at location $r$.

The potential $V$ can be solved by setting the boundary conditions according to the geometry of the stimulating electrodes. The tACS-elicited currents inside the head follow the Ohm’s law:

$$J(r) = \sigma(r)E(r) = -\sigma(r)\nabla V(r),$$

(3.4)

where $E$ is the tACS-driven electric field.

As can be predicted, the tACS-elicited currents are most pronounced in the well-conducting scalp [151]. According to simulations, the current density in the brain is only one tenth of that in the scalp [151]. Also inside the skull, the currents tend to travel mostly through the well-conducting CSF rather than in the cortical volume [151], further decreasing the effect in ROI. However, part of the current travels through the gray matter affecting cortical activity. According to modelling studies, the resulting current density in the cortical ROI is around $0.1 \text{ A m}^{-2}$ when using typical tACS stimulation intensities [142, 51, 43, 151]. Corresponding results
have been obtained when measuring invasively the intracranial fields in a non-human primate [165]. In Fig. 3.3, an example of a tACS-driven current density distribution inside the brain is visualized.

![Figure 3.3. Modelling the tACS-driven currents inside the brain, when the stimulating electrodes are placed above the occipital lobe and the vertex of the head. The simulation shows that, overall, the currents in the cerebrum are weak. The strongest currents are generated in the region directly under and between the stimulating electrodes. Figure reprinted with permission [151].](image)

### 3.3.2 Cortical effects of tACS

Similarly as with TMS, tACS-caused electric fields hyperpolarize and depolarize nerve structures in the brain [192]. However, since most of the driven current travels through the scalp and CSF, and only small current densities are generated in the brain [151], the depolarization resulting from tACS is not strong enough to launch action potentials on its own. The intracranial current density is weak also because of the low stimulation intensities that are used to minimize the adverse effects, such as dizziness, skin sensations, and phosphenes [139]. On the other hand, electrical stimulation can be switched on for considerably longer period
of time than a single TMS pulse. A typical tACS session can last from a few to tens of minutes [86] during which the probability for a neuron to launch an action potential oscillates between elevated and depressed states according to the phase of the tACS current [192]. Animal models show that even weak intra-cranial electric fields, having magnitudes of \(0.1–1 \text{ V m}^{-1}\), were able to synchronize neural firing to the phase of the applied sinusoidal current [63, 167]. These results are supported by a neural simulation study [97]. However, a recent invasive study using typical tACS intensities failed to show any tACS-elicited modulation in the cortical activity in human [121].

### 3.3.3 tACS instrumentation

The instrumentation needed for performing tACS is simple. Basically, a sufficiently powerful current source is needed to drive about \(1 \text{ mA}\) current [251] through the head, which has with the electrode contacts a load resistance of a few kiloohms [83]. Despite the simple circuitry, practically all tACS studies use commercial stimulation modules [236, 208, 147, 31]. These devices can be used on their own to drive simple current waveforms, but some models can be connected to an external signal generator allowing practically arbitrary tACS waveforms.

The stimulator devices are connected to the scalp of the subject via conductive pads. Often the pads are inserted inside sponges, which are dipped into saline solutions and attached to the desired locations with rubber bands [251]. A more robust and flexible way to prepare the contact between the scalp and stimulating electrodes is to use conductive gluing paste [241]. Most commonly, only two electrodes are attached on the scalp, but in principle more electrodes could be used to target more accurately the generated electric fields to the desired brain region [51, 43].

### 3.3.4 Applications of tACS

tACS has been used mainly in brain research to probe the causal connections between different brain oscillations and cognitive functions [172]. For instance, the roles of different oscillation frequencies on the excitability of M1 have been investigated in several studies [6, 182, 144]. Among many other topics, tACS has been used to study, e.g., the importance of
slow-frequency oscillations in memory consolidation [137] and encoding [109], as well as probing the roles of different frequency bands in tactile sensation [58]. In addition to studying the functional roles of different brain rhythm frequencies, also the importance of the phase in the neuronal oscillations has been studied with tACS [183, 149].

Currently, there are no well-established clinical applications for tACS although the technique has been suggested as a potential therapeutic tool for treating Parkinson’s disease and schizophrenia [22, 172]. Note that tACS should not be confused with electroconvulsive therapy [2], which uses significantly higher currents, commonly as high as 0.8-A pulses [136, 45].

The combination of tACS with concurrent EEG is still in its infancy. Partially, this is due to the tACS-induced currents in the scalp that are considerably stronger than those resulting from neuronal activity. There are only a few studies that have attempted to measure the concurrent cortical effects of tACS directly with EEG [80, 239].

### 3.4 Electroencephalography (EEG)

EEG quantifies the electric brain activity by measuring the potential differences across the scalp of the subject [152]. The first human-EEG results were reported in 1929 by Berger [16], who probed brain oscillations by measuring the potential difference between the forehead and the occiput with a galvanometer. Since then, EEG methodology has obviously evolved considerably. The modern EEG recordings involve the subject wearing an elastic cap that holds several small electrodes. The electrodes, which are spread across the head, are brought to contact with the scalp using conductive paste. By measuring the voltage values between the reference and the other electrodes on the head, it is possible to infer the likely origin of the observed brain activity or study changes in the cortical state. EEG is widely used both for various research and diagnostics applications [152], e.g., for defining the approximate location of the epileptogenic zone [157] or for studying how the brain responses to different stimuli e.g., [146]. Right from the start, including the pioneering work by Berger, EEG has been used to investigate the functional roles of different
brain oscillations [16, 110, 68, 228] typically including delta (0.5–3.5 Hz), theta (3.5–7 Hz), alpha (8–13 Hz), beta (15-25 Hz), and gamma (30–70 Hz) frequencies [243].

3.4.1 Physical basis of EEG

Neuronal ion flows establish electric fields in the head that cause ohmic currents to flow both in the brain and in the surrounding tissues [135]. These electric fields can be detected by measuring potential differences on the scalp. Although both the action-potential and the synapse-related ion flows generate ohmic currents, EEG is thought to mostly reflect the post-synaptic currents in the pyramidal cells [163]. This can be explained by the difference in the post-synaptic-current and action-potential generated extra-cellular electric fields. From a distance, post-synaptic currents appear as dipolar sources [163] whereas action potentials can be described as quadrupoles [247]. With distance $r$ from the source, the electric field generated by a quadruple source falls off as $r^{-4}$ whereas the dipolar field falls off as $r^{-3}$ [70]. Furthermore, since action potentials are short-lived, the temporal summation of currents in a nerve population is less probable than the summation of post-synaptic currents [73]. Even in the case of post-synaptic currents, at least thousands of synchronous post-synaptic sources, with similar orientation, are needed to result in a detectable EEG response. There have been studies recording the spiking activity of neurons with EEG [8, 193] but this requires a somewhat specialized methodology.

On a macroscopic scale, the total current density in the head at $r$ can be written:

$$ J_{\text{tot}}(r) = J^p(r) + J^v(r) = J^p(r) + \sigma(r)E(r), \quad (3.5) $$

where $J^p$ is the primary current corresponding to the post-synaptic activity in synchronous cell populations, $J^v$ is the ohmic current density driven by the primary current, and $\sigma$ is the conductivity [249, 248]. The corresponding (voltage) measurement, $y_s$, by EEG channel $s$ can be written as a line integral:

$$ y_s = \int_{r_a}^{r_b} E(r') \cdot dl(r'), \quad (3.6) $$
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where \( r_a \) and \( r_b \) are the locations of the electrodes \( a \) and \( b \), respectively, which form the EEG channel \( s \).

The recorded EEG signals depend on the primary-current distribution and the conductivity profile of the head [13, 215], which together determine the measured electric field. Before reaching to the scalp, the primary-current-driven volume currents have to pass through the CSF and skull. Because CSF is well conducting and skull is poorly conducting, most of the neuronal elicited volume currents tend to stay within the intracranial cavity [216]. This means that the electric field on the scalp is relatively weak, and therefore, the amplitudes of the observed signals have relatively low signal-to-noise ratio (SNR) in contrast to invasive recordings. Another well-known characteristic of EEG signals is that the neuronal potential patterns are smeared by the large contrast in the conductivity between the skull and scalp, and the brain [245]. As a result, the neuronal EEG signals are heavily correlated across the channels.

3.4.2 Challenges in combining brain stimulation with EEG

Combining EEG with simultaneous TMS or tACS is challenging for several reasons. In addition to affecting the brain in ROI, the generated electromagnetic fields couple easily also with extracranial tissues and the recording instruments. This results in number of noise and artifact signals\(^1\) with various spatiotemporal characteristics. Thus, multiple measures are needed to tackle the TEBS-caused EEG disturbances.

The time-varying magnetic field of TMS induces voltages in the electrode leads, which may saturate the amplifier for hundreds of milliseconds [94]. The first attempt to combine EEG with concurrent TMS was performed by Cracco et al. in 1989 [36] who measured the transcallosal responses to TMS that was targeted to the central sulcus. However, because of the coupling of the changing magnetic field with the EEG leads, only responses approximately 10 ms after TMS were observed.

To prevent amplifier from saturating, specialized EEG amplifiers should

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\(^1\)The distinction between an artifact and noise is to some extent arbitrary. After all, they both hinder the observation of the signals of interest. In the EEG literature, systematically occurring, short lived disturbances are commonly referred to as artifacts, whereas the less well-defined signal components of stochastic nature are often called noise.
be used. The first TMS-compatible EEG amplifiers were switched off for a few milliseconds after the TMS pulse [237]. With the help of this recording instrumentation, on 1997, Ilmoniemi et al. were able to record early ipsilateral reactions to TMS pulses that were delivered to M1 [95]. Other ways to tackle the TMS-induced artifacts are to use slew-rate limited preamplifiers [96] or EEG devices with a high dynamic range to prevent the amplifiers from saturating [20]. The high-dynamic-range amplifiers usually also offer an option for very high sampling frequency, which helps in modeling and subtracting the TMS-induced artifact in the offline signal processing. The problem with this fitting-and-subtracting approach is that the high-amplitude artifact should be modeled extremely accurately to ensure that no significant residual artifacts remain after the artifact-rejection.

In addition to using the appropriate EEG amplifiers, using TMS-suitable electrodes helps in minimizing the induced artifact. Instead of traditional ring electrodes, C-shaped or pellet-type electrodes should be favored. Smaller electrodes, such as pellet electrodes, also suffer less from heating when compared to standard ring or disk electrodes [201]. This improves the subject’s safety as well as decreases the thermal voltage noise [94]. Another way to decrease the electrode heating is to choose electrode material with sufficiently low conductivity. Today, most of the TMS–EEG measurements are done with Silver/Silver Chloride electrodes [164, 32, 57, 55, 185, 158], which have been found to be TMS compatible.

Another significant challenge in combining TMS with EEG is the presence of the high-amplitude, exponentially decaying artifacts, introduced immediately after the stimulating pulse. These artifacts are likely to reflect the capacitive properties of the skin [100] and the skin–electrode interface [93]. Most efficient approach to decrease the amplitude of the decaying artifacts seems to be a careful electrode preparation, and thus minimizing the electrode-skin contact impedance. Also modeling and subsequent subtraction of the capacitive artifact has been used [105, 30].

Similarly as in the brain, TMS also induces an electric field in the scalp. As was discussed in Section 3.2.1, the strength of the TMS-induced electric field decreases rapidly as a function of distance from the coil. Because the intensity of TMS is adjusted according to the cortical excitation threshold, the resulting stimulation intensity in the extracranial tissue is
sufficient to activate also the scalp nerves and cranial muscles [94]. When recording EEG, this can result in large muscular artifacts that considerably hamper the use of TMS–EEG. We addressed this problem in Publications I and III, and therefore, muscle artifacts are discussed in more detail in a dedicated section.

In addition to the cortical reactions to the induced electric field, TMS can cause also unwanted neuronal responses through indirect mechanisms. For instance, during the current pulse, the mechanical forces that are exerted to the coil cause loud clicks. Without appropriate precautions, these clicks evoke clear EEG deflections. In the worst case, the clicks might even damage the hearing of the subject. A common approach to deal with the auditory-evoked response is to play masking noise to the subject’s ears while also blocking the TMS click with headphones [224]. However, this is not necessarily sufficient to block the auditory artifact completely, as the acoustic vibration of the coils can be transmitted to the ear also by bone conduction [155]. To minimize the bone conduction, an addition of thin foam layer between the scalp and the coil has been suggested [194]. The auditory-evoked artifact can be also taken into account with an additional sham-stimulation condition [40].

It is also possible that TMS elicits somatosensory-evoked responses. Often, a TMS pulse causes a clear sensation on the scalp. In addition, when stimulating M1 with TMS, the subject can notice the twitches in the peripheral muscles. The significance of somatosensory evoked artifacts is not studied thoroughly but the current consensus is that they are not a major problem when performing TMS–EEG [94, 194].

The indirect neuronal responses to TMS, such as the click- and sensation-related artifacts, can be challenging to reject in post signal-processing, because they are likely to result in EEG signals very similar to those caused by the TMS-induced electric field. The best way to deal with the neuronal noise sources is to avoid them already at the recording stage by carefully planning the measurements.

The main problem when combining tACS with EEG are the tACS-driven currents in the scalp. Since the scalp is relatively well conducting compared to the poorly-conducting skull, the current density is very high close to the recording electrodes [151]. The problem is emphasized by the reciprocity principle [82]; a bipolar voltage measurement, such as EEG,
most sensitive to those currents that would be generated if the voltage would be asserted across the recording electrodes [206]. In other words, EEG electrodes couple very easily with the electric fields generated by the tACS electrodes. To allow the offline rejection of the tACS-induced artifact, the EEG amplifier should have a sufficiently high dynamic range. tACS-induced EEG artifacts were studied in detail in Publications V.

In addition to the stimulation-specific challenges, TMS–EEG and tACS–EEG data are both prone to suffer from similar problems as plain EEG: e.g., line noise, eye movement, and badly connected/broken electrodes. Especially these random disturbances, as well as the TMS-pulse related decaying artifacts were addressed in Publication IV.

3.4.3 TMS-evoked muscle artifacts in EEG

A major challenge hindering the use of TMS–EEG has been the TMS-evoked muscular artifacts. In addition to intracranial electric fields, the TMS pulse induces a similar electric field also in the extracranial tissues. The magnitude of the extracranial electric field is at least comparable to the electric-field magnitudes used in electrical muscle stimulation [130, 143], and thus, TMS is likely to activate the muscle fibers of the scalp [94].

The largest scalp muscles are the occipitofrontalis, and the temporalis muscle [232]. Placing the TMS-coil to the vicinity of these muscles poses a significant risk for EMG contamination in the EEG data. It has been demonstrated that the magnetic stimulation of temporal cortical areas, such as Broca’s area and dorsal premotor cortex results in large initial EEG deflections [118] similar to, for instance, motor-evoked potentials measured in the peripheral muscles with EMG (see, e.g., [238, 59, 111]). However, prior to Publication I, a systematic mapping of the muscle-artifact sources was missing.

Muscular activity is manifested in EEG on a broad frequency spectrum of approximately 0–200 Hz [69], heavily overlapping with the typical frequency content of neuronal EEG signals [69]. The same holds also for the TMS-evoked muscle twitches [133]. Thus, a conventional low-pass filtering is not sufficient for removing these muscle artifacts. On the other hand, the neuronal EEG signals are most pronounced below 100 Hz [26].
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This feature was utilized in Publication III, where we developed a signal-
processing method to reject the TMS-evoked muscle artifacts in EEG.

The offline rejection of muscle artifact from the measured signal is chal-
lenging for a couple of reasons. First of all, the artifacts can have ampli-
tudes that are several orders of magnitude larger than those of the TMS-
evoked neuronal EEG responses. Hence, even a relatively small residual
artifact remaining after signal processing can still severely contaminate
the resulting EEG signals. Furthermore, muscle artifacts are correlated
across the EEG channels. Even if an EEG channel if far from the activated
muscle, it can still contain a considerable amount of EMG signals. Finally,
muscle artifacts and neuronal EEG signals are heavily overlapping both
in the frequency and in the temporal domain. Because the perfect rejec-
tion of muscle artifacts in the offline signal-processing can be difficult, we
tested in Publication I how theses disturbances could be avoided already
online by choosing the most appropriate stimulation parameters, meaning
the location and orientation of the coil as well as the TMS intensity.

3.4.4 Previous EEG-analysis approaches to remove TMS and
tACS-related artifacts

There have been several suggestions for removing the TMS- and tACS-
related EEG artifacts in the offline data analysis. The use of independent
component analysis (ICA) for rejecting the TMS-evoked muscle responses
has been studied in several papers [118, 85, 196]. The problem with ICA is
that heuristic knowledge is needed to identify certain independent compo-
nents as artifactual. Furthermore, the artifact components might not per-
fectly fulfill the assumption that they are statistically independent from
the components with neuronal origin. This easily leads to overcorrection
at the early latencies, when the muscle artifacts are present.

Principal component analysis (PCA) [223] and a closely related method
called signal-space projection (SSP) [134] have also been applied to sup-
press the muscle artifacts. SSP was also used in Publications II and V,
and thus will be discussed more thoroughly in Section 3.5.2. The problem
with these PCA-related methods is that they may distort significantly the
appearance of the remaining EEG data. This can make the subsequent
interpretation of the data difficult. The problem, however, can be resolved
easily, as we describe in Publication III. An additional problem with the
simple PCA approach [223], where a few main components of the whole data are completely removed, is that also a considerable amount of brain data of interest can be lost if the muscle artifacts and the neuronal EEG deflections are correlated.

In addition to muscle artifacts, there are several other TMS-related EEG artifacts whose removal has been discussed in the literature. It is common to find at least a few corrupted channels in the TMS–EEG measurements, e.g., due to polarized skin–electrode interfaces and other transient disturbances. A popular approach to deal with these artifacts is to visually inspect the data to reject and replace the bad-quality channels using spatial interpolation, see, e.g. [101, 27, 7]. In Publication IV, we present an alternative approach that optimally utilizes all the gathered data, and requires less time and heuristic information than the visual inspection and rejection.

So far, there are only a few papers that have attempted to analyze EEG data that were collected concurrently with tACS [80, 239]. In these papers, the authors computed an artifact template, which was fitted to the data and subsequently rejected. The main problem with this approach is that if the entrained brain oscillation has the exactly same frequency with the stimulating sinusoidal wave, then at least the component of the signals of interest that is in phase with tACS will be subtracted. Furthermore, because the artifact can be several orders of magnitude greater than the neuronal signals, even a small relative error in the artifact template can result in significant residual artifact.

An alternative approach to tackle the tACS artifact would be to design a spatial filter that would project out the artifact patterns in the EEG. For instance, spatial beamforming techniques have been suggested to remove the tACS artifact in magnetoencephalography (MEG) signals [212, 150]. In Publication V, we studied the use of a spatial filter, in this case SSP, to suppress the tACS-induced EEG artifacts.

3.4.5 Measuring cortical responses to TMS with EEG

The conventional approach to study the cortical effects of TMS has been to deliver single TMS pulses repeatedly with randomized inter-stimulus intervals and compute the average EEG response over the resulted TMS–
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EEG trials [95, 114, 20, 124, 56, 52, 225]. Using this paradigm, we have learned, for instance, how the effective connectivity changes on average in different sleep and awake states [138, 153] or how cortical reactivity might be modulated in different mental conditions [156, 89].

However, surprisingly little EEG study has been done to quantify how a single TMS pulse perturbs and modulates the ongoing spontaneous brain activity. It has been shown that a single TMS pulse enhances the natural cortical rhythms at the stimulation site [198] and that by delivering a pulse train with the frequency of interest it is possible to entrain the cortical oscillations at the corresponding frequency [229].

Recently, an interest has emerged over the so called closed-loop brain stimulation, meaning that the stimulation parameters are chosen according to concurrent recordings, such as EEG [17, 257, 258]. In its simplest form, this might mean delivering TMS at the moment of the most appropriate brain state, but in principle also other parameters such as intensity, stimulation location, or the TMS-pulse shape could be tuned according to the current neuronal activity.

To maximize the effectiveness of closed-loop brain stimulation it is important to understand two aspects: First, how does the current brain state change the effects of TMS [57, 55], and second, how does TMS perturb the ongoing brain activity, as this might affect the cortical reactivity to the following TMS pulses. By analyzing solely the average EEG responses it is not possible to quantify the immediate TMS-elicited changes in the current brain state. In Publication II, we developed measures to quantify these effects from trial-level TMS–EEG data.
4. Methods

In this chapter, the main methodological aspects of the five publications included in this Thesis are discussed. First, the main components of the applied recording equipment are reported, after which the key theoretical concepts behind the developed signal-analysis tools are explained. In particular, the use of the linear model for the EEG data is elaborated.

4.1 Recording equipment

4.1.1 TMS–EEG measurements

All the TMS–EEG data presented in the Thesis, i.e., Publications I–IV, were recorded using the same equipment. TMS was delivered using the Nexstim eXimia system (Nexstim Plc, Helsinki, Finland) with a figure-of-eight coil. The TMS-evoked EEG was recorded with the TMS-compatible Nexstim eXimia EEG system, which uses sample-and-hold circuitry to block the TMS-induced electrical artifact [237]. The EEG system had 60 electrodes on the scalp in a 10–20 montage.

For accurate targeting of TMS to the desired cortical location, the Nexstim eXimia Navigated Brain Stimulation (NBS) system was used. NBS uses an infrared camera to monitor the location and orientation of the TMS coil in relation to the subject’s brain by tracking the positions of the infrared markers in the coil and in the head tracker. The whole navigated TMS–EEG system is illustrated in Fig. 4.1.

For a more precise description of the recording parameters used in different studies, see Publications I–IV.
Methods

Figure 4.1. TMS–EEG equipment. With the help of the infrared-tracking system, the NBS software visualizes in real time the stimulation location and an estimate of the TMS-induced electric field on the subject’s cortical surface.

4.1.2 tACS–EEG measurements

In Publication V, we studied the offline removal of the tACS-induced EEG artifact. For this, two separate studies were performed. In the first study, we built a tACS–EEG phantom head to quantify reliably the success of the tested artifact-rejection methods. The phantom included a neuronal current source, EEG-recording system, and two terminals for driving the tACS current. The structure of the phantom is illustrated in Fig. 5.5 and described in detailed in Publication V.

In addition to the phantom study, a human tACS–EEG study was performed to further validate the performance of SSP in suppressing the tACS-induced EEG artifact. In the human study, we recorded EEG simultaneously while stimulating the occipital region of the head. For EEG, we used a 24-channel active EEG system (ActiChamp, Brain Products, Munich, Germany). tACS was delivered via two rubber electrodes, which were attached using sticky adhesive paste. For tACS, a battery-powered, tACS-capable stimulator system (DC stimulator plus, Eldith, Neuroconn, Ilmenau, Germany) was used. For more detail, see Publication V.
4.2 Signal and current spaces

As was discussed in Section 3.4.1, EEG recordings are mainly sensitive to synchronous post-synaptic currents in pyramidal cell populations [152]. The information processing in the brain is fundamentally based on electric signals, namely action potentials. As each post-synaptic current depends on the weighted sum of the action potentials arriving to the synaptic terminal [152], post-synaptic currents can be interpreted as roughly linear, low-dimensional projections of the action potentials. Thus, in the context of EEG, the post-synaptic currents can be thought to define the brain state at the finest possible scale, since most of the current EEG devices are not sensitive to high-frequency phenomena, such as action potentials.

We can now define the current space that holds all the possible primary current distributions (EEG-relevant brain states) [72]. The state of the brain at a certain instant of time can be characterized by a current vector. Each element of this vector describes the primary-current density in the indexed location to one of the three Cartesian directions, i.e., three components is needed to define the primary current at one location. Because the number of points in any three-dimensional volume is infinite, the dimension of the current space and the current vector is infinite as well. As the state of the brain changes, so does the length and orientation of the current vector in the current space.

Each of the components of the primary-current vector produces a specific electric field in the head. The voltage measurements of EEG can be described in terms of line integrals of these fields in the scalp (See Eq. (3.6)). Because of the linearity of line integrals, we can express the EEG measurement simply in terms of the primary-current distribution [74]:

\[ y_i = \int l_i(r) \cdot J^P(r) dV, \]  

(4.1)

where \( y_i \) is voltage measured by EEG channel \( i \) due to primary current distribution \( J^P(r) = [J^P_x(r), J^P_y(r), J^P_z(r)] \), and \( l_i(r) \) is the so-called lead field that defines how sensitive channel \( i \) is to different orientations of primary-current at different locations in the brain.

The exact shape of the lead field depends on the locations of the elec-
trodes forming the EEG channel and the geometry and the conductivity profile of the head. The term "lead field" refers to the reciprocity principle [82], which states that the sensitivity profile of a voltage-measuring electrode pair can be found by calculating the electric field that would be formed if a current would be driven through the same electrode pair [206]. A visualization of an example EEG lead field is given in Fig. 4.2.

![Figure 4.2](image)

**Figure 4.2.** The lead field of a typical EEG channel. The red and black dots show the locations of the two electrodes forming the EEG channel. The colormap shows how the relative sensitivity of the studied EEG channel varies as a function of the location of the post-synaptic current on the cortical surface. The small arrows indicate the directions of post-synaptic currents that would be most easily detected by the EEG channel.

We can now define the signal vector $y = [y_1, y_2, \ldots, y_S]$, which contains all measurements by EEG channels $i \in \{1, \ldots, S\}$. The signal space is an $S$-dimensional space that can explain any measurement of an $S$-channel EEG system. From Eq. (4.1), we can see that an EEG measurement is actually an $S$-dimensional linear projection of the electric brain state lying in the current space. Thus, by observing changes in EEG we have a direct, albeit a low dimensional, readout of the electric state of the brain (See Fig. 5.2 for conceptual illustration).
An interesting question is what is the natural state of the brain in different conditions, and how is this state affected by a TMS pulse that abruptly activates an entire neuron population. It would seem natural to assume that the TMS-evoked primary-current distribution is less probable than the states that are spontaneously occupied. This could be manifested as a sudden shift in the current vector but also possibly in the EEG, which provides a direct projection of the post-synaptic activity. The current and signal-space formalisms were applied in Publication II, where we studied transient effects to TMS on the current brain state. The concept of lead field is also fundamental in understanding the methods in Publications III and V.

4.3 Modeling neuronal EEG signals

Often the goal of EEG experiments is to locate the brain activity at different time instants with respect to the onset of a stimulus or determine the anatomical origin of some neuronal oscillation. To determine which brain areas were active, we first need to solve the forward problem: What are the sensitivity profiles, i.e., the lead fields of different EEG channels? Often we have some prior information about the anatomical origin of the EEG signals of interest. Thus, instead of the whole volume of the brain, only ROI (e.g., the cortical surface) is included in the analysis. From a distance, the net volume currents resulting from synchronous post-synaptic currents in a group of neurons appear similar to those volume currents generated by a dipolar current source [135, 163]. The dipolar current source is a sufficiently accurate model for the net post-synaptic activity originating in a cortical column with a millimeter-scale diameter [163]. Thus, a natural way to model the primary currents in continuous medium is to discretize ROI with sufficiently small surface or volume elements and place a current dipole in each element. By calculating the EEG-signal amplitudes resulting from a single active current dipole in one element, we can form the approximate lead fields that map the sensitivities of different sensors to different dipolar sources lying in the chosen ROI. The discretization is practical for computational purposes as Eq. (4.1) can be written as a matrix product.
The critical decisions in the forward computations are the choice of ROI and to which accuracy the conductive properties of the head are modeled. A common choice for ROI in EEG analysis is the surface defining the gray–white matter interface [95, 108, 114, 138, 4]. The head is usually described in terms of three different conductivity compartments: the brain, the skull, and the scalp [216]. It is recommended, however, to include the effects of CSF in the model [216]. The geometry of the head model can be either simplified or follow more realistically the subject-specific anatomy, which requires the use of MRI or computed tomography (CT) [38]. A typical example of a simplified geometry is the concentric three-shell sphere [206].

After discretizing the current space, the recorded EEG data $Y$ can be written as follows:

$$Y = LJ + N,$$

where $L$ and $J$ are the approximate lead-field and source matrices, respectively, and $N$ is the noise. The element $L_{i,j}$ corresponds to the sensitivity of sensor $i$ to neural equivalent source $j$, whereas the element $J_{j,k}$ is the activity of the equivalent source $j$ at time $k$. In the next section, we shall discuss one approach to estimate the solution to the inverse problem: given $Y$ and $L$, what is $J$?

4.4 Minimum-norm estimate and signal-space projection

The inverse problem of EEG is to estimate the primary current distribution based on the voltage measurements on the scalp. The problem will always be ill-posed; there are infinitely many solutions that would produce the same EEG measurement [82]. Because the number of EEG electrodes is very limited with respect to the dimensionality of the primary currents, the problem is even more challenging [92]. To circumvent the non-uniqueness of the inverse problem, we must estimate the primary-current distribution by using our prior knowledge to set the most appropriate constraints that the possible solutions must satisfy. One such approach is to find the minimum-norm estimate (MNE) [72, 74].

The idea of MNE is to look for the current distribution producing an
EEG output with minimal expected difference to the actual recording. Simultaneously, the norm of the current estimate is minimized [72], meaning that the recorder data would be generated with the smallest possible net primary-current amplitude (least energy). The MNE solution will be a linear combination of the lead fields. This means that the estimate is limited by our measurement system. Without some additional information, we cannot make conclusions of the primary-current components that are invisible for the sensors. Furthermore, those brain regions that are best seen by the sensor array are emphasized in the estimate.

MNE seems, perhaps, more intuitive if we first consider only a single recording channel. We place a pair of electrodes to arbitrary points on the head and start recording. Suddenly, we observe a large deflection in the studied EEG sensor. It seems more reasonable to assume that this deflection was generated by a low-amplitude primary-current source in the vicinity of the channel, rather than a high-current-amplitude source in a remote location, almost invisible for the sensor. MNE can be thought as a mathematical generalization of this idea for multi-channel recordings.

Mathematically, MNE can be written as a weighted sum of the lead fields of all the channels:

$$\hat{J} = \sum_i w_i l_i,$$

where $\hat{J}$ is the current estimate and $w_i$ is the channel-specific coefficient, corresponding to lead field of channel $i$, $l_i$. The coefficients are chosen such that both $|L\hat{J} - Y|^2$ and $|\hat{J}|^2$ are minimized. In matrix notation, the solution can be written:

$$\hat{J} = L^T (LL^T)^{-1} Y.$$

As indicated by Eq. 4.2, measured signals always contain some noise. Because of the ill-posed nature of the problem, the noise tends to be amplified in the current estimate. To control the amount of noise leaking to $\hat{J}$, some sort of regularization is applied [74]. For instance, singular-value truncation [66] or Tikhonov regularization [230] can be used. An appropriate regularization level can be determined, e.g., by using the information of SNR [123], Morozov’s discrepancy principle [145], or by setting an acceptable goodness-of-fit level [218] that the MNE must achieve.
Methods

A nice feature of MNE is that it reconstructs the measured signal well. This property can be used to extrapolate data onto virtual measurement channels [162, 25], such as channels that were not used in the actual recordings or that were rejected from the analysis due to noise contamination.

If we know the lead field of sensor $s$, $l_s$, the signal in the corresponding sensor can be estimated as

$$\hat{y}_s = l_s \hat{J},$$

where $\hat{y}_s$ is a row vector containing the estimated time course in sensor $s$. The accuracy of the extrapolation depends on how well the cross correlations between the lead field of the extrapolated channel, and the lead fields of the other channels are known [162]. The MNE extrapolation was the basis for the noise-suppression algorithm developed in Publication IV.

A conventional way to analyze EEG is to study signals in specific channels based on their locations with respect to different brain regions. A slightly more sophisticated approach, commonly applied by clinicians, is to transfer the measured data onto a more informative reference, such as bipolar, see e.g., [114], or source reference systems [88]. Essentially this means subtracting a certain linear combination of all EEG traces from each electrode-specific EEG signal. As a result, a set of new virtual channels and the corresponding EEG traces are formed. Mathematically, the signal in virtual channel $s$ can be written as

$$y'_s = y_s - \sum_i p_{s,i} y_i,$$

where $p_{s,i}$ are the $s$-specific weights for the original channel traces. Eq. (4.6) can be written in matrix notation for the whole data $Y' = PY$, where $P$ is the projection matrix, which has the weights $p_{s,i}$ as its elements.

The virtual channels have more desired sensitivity for the brain ROIs. Thus, these virtual channels are described by new lead fields that are, correspondingly,

$$L' = PL.$$

Signal-space projection [234] generalizes this approach. If we do not know in advance the virtual channels that have the optimal sensitivity for
the phenomena of interest, we can estimate the projection matrix from the measured data. For instance, if we know that the phenomena of interest take place in a certain time interval or in some frequency range, we can select this sub part of data $Y_{\text{sub}}$ and use it to estimate the optimal virtual channels.

We can write $Y_{\text{sub}}$ in terms of singular-value decomposition (SVD). $Y_{\text{sub}}$, having $S$ number of sensors and $N$ samples, can then be written

$$Y_{\text{sub}} = U\Sigma V^T,$$  \hspace{1cm} (4.8)

where $U$ and $V$ are $S \times S$ and $N \times N$ unitary matrices, respectively, and $\Sigma$ is a diagonal matrix containing the singular values. The column vectors of $U$ form an orthonormal basis for the whole signal space. As the first $k$ column vectors explain most of the variance in $Y_{\text{sub}}$, the same set of vectors can be used to construct a projection matrix $P = U_k U_k^T$ that projects the data onto a set of virtual channels that optimally record the phenomena of interest.

An important application of SSP is artifact rejection. If we are able to define $Y_{\text{sub}}$ such that it mainly contains the artifact signals, the resulting $P$ projects the original data onto a signal subspace that optimally measures these artifacts. Then correspondingly, to avoid these artifacts, the original data $Y$ should be projected onto the orthogonal signal subspace:

$$Y' = P_\perp Y,$$ \hspace{1cm} (4.9)

where $P_\perp = I - P$, $I$ being an identity matrix. SSP can be easily taken into account in the inverse estimation; $Y'$ and $L'$ can be directly substituted to Eq. (4.4). If one wishes to reconstruct the original channel traces from $Y'$, then the MNE-extrapolation technique (Eqs. (4.4) and (4.5)) can be applied. This reconstruction step is important if one wishes to analyze the cleaned data visually, as it is well known that the cleaned signals in the SSP-generated virtual channels may seem distorted. This apparent distortion is a consequence of the new lead fields (See Eq. (4.7)), which do not correspond to the physical locations of the electrodes, and thus, are less intuitive. However, through the MNE extrapolation, the information remaining after the artifact rejection can be presented in a more meaningful way.
Methods

SSP was used in Publications III and V to suppress TMS and tACS-related EEG artifacts, respectively. In Publication III, we concentrated on the TMS-evoked muscle artifacts. As was discussed in 3.4.3, the muscle artifacts overlap with neuronal EEG activity in the frequencies below 100 Hz. However, unlike the neuronal EEG signals, the muscle artifacts also have major components at higher frequencies. Thus, we first high-pass filtered the data from 100 Hz to reject the cortical responses to TMS. From this sub part of the data, we then estimated the SSP operator that projected the data onto the artifact-free signal subspace. In Publication V, the artifact-suppression operator was estimated from the tTACS-artifact template, which was obtained by averaging the EEG signals across the recorded stimulation periods. In both publications, the projected signals were finally reconstructed in the original EEG channels using MNE extrapolation.

4.5 Estimate for noise-free EEG signals

In EEG, the brain activity is quantified by recording the potential difference simultaneously from several locations on the scalp. Because of the conductive properties of the scalp and the skull, the resulting EEG signals will be considerably correlated. Thus, by looking at the signal measured by some arbitrary channel, we already obtain a lot of information of the data measured by the other EEG traces, and vice versa.

EEG recordings always contain some noise. Because of the correlated nature of EEG, it turns out that the optimal estimate for noiseless signal in some channel \( s \) is not the trace of \( s \) itself, but a linear combination of all the recorded EEG signals.

Let us consider the measured data depicted in Eq. (4.2). We seek for an optimal estimate for the noiseless measurement in sensor \( s, \bar{y}_s \). To achieve this, we look for the coefficients \( \hat{w} = [\hat{w}_1, \ldots, \hat{w}_S]^T \), \( S \) being the number of all sensors, that minimize the mean squared error (MSE), i.e.,

\[
\hat{w} = \arg \min_{w \in \mathbb{R}^S} \mathbb{E}\left( (\bar{y}_s - \sum_{i=1}^S w_i y_i)^2 \right). \tag{4.10}
\]

Provided that we have a sufficient number of samples in time, or across trials, the expectation value can be approximated as the sample mean.
Thus, we look for the weights minimizing $|\bar{y}_s - \hat{w}^T Y|^2 / T$, where $\bar{y}_s$ is a row vector containing the noiseless signal measured by sensor $s$ and $T$ the number of samples in $\bar{y}_s$. The function to be minimized is a parabola whose minimum can be found at the zero of the derivative:

$$
\frac{d}{dw} |\bar{y}_s - \hat{w}^T Y|^2 / T = 0
$$

$$
\rightarrow \hat{w} = C^{-1} r_s,
$$

where $C$ is the correlation matrix of the data $Y$, and $r_s$ is the cross correlation vector between $Y$ and $\bar{y}_s$ [79].

By observing from Eq. (4.2) that $\bar{y}_s$ corresponds to $l_s J$, and by assuming that the noise and the primary currents are not correlated, we can write the data correlation matrix and the cross correlation vector for EEG measurements as follows:

$$
C = YY^T = LC_J L^T + C_N
$$

$$
r_s = Y\bar{y}_s^T = LC_J I_s^T,
$$

where $C_J = JJ^T$ and $C_N = NN^T$ are the primary-current and noise correlation matrices, respectively. Thus, if $C_J$ and $C_N$ are known, the optimal estimate for the noiseless sensor signal (in the minimum MSE sense) in sensor $s$ is given by:

$$
\hat{y}_s = l_s C_J L^T (LC_J L^T + C_N)^{-1} Y.
$$

The weights correspond to Wiener filtering [246], which is, by definition, a linear estimator that minimizes the MSE [79].

The result in Eq. 4.13 has a few important implications regarding the common analysis practices in the EEG literature. A very popular technique to deal with measurement noise is to visually inspect the data and to categorically reject or accept "bad" or "good" data segments, respectively. However, according to Eq. 4.13 there are no strictly "bad" or "good" channels. In theory, SNR in all EEG signals should improve due to Wiener filtering. Wiener estimation will also take care of the "bad" channels, as the truly noisy and unreliable channels will be practically completely interpolated based on the other channels.

Eq. 4.13 can be considered as a mathematical formalization of the visual inspection; the channels or trials are cross-validated, and if a data seg-
ment differs considerably from the other measurements it can substituted with an interpolation. The use of Wiener filtering, however, enhances this process substantially and requires no heuristic knowledge.

As already hinted, Eq. 4.13 can be applied directly to other dimensions than sensors, e.g., trials, as long as the correlation matrices are known. In principle, the data could be cross-validated, for instance, across the subjects to correct the outliers. The Wiener estimator was utilized in Publication IV where we developed an algorithm for automatically cleaning EEG data from various noise components. In the particular version of Wiener filtering, the noise-correlation matrix was estimated through an iterative cross-validation scheme and the primary currents were assumed to be independent and identically distributed. In the same work, we also demonstrated how Wiener filtering can be performed in a data-driven way if the data- and the cross correlation matrices are not known.
5. Summary of Publications

In this chapter, the main results of the five included publications are briefly described.

5.1 Publication I: The effect of stimulus parameters on TMS–EEG muscle artifacts

The purpose of this publication was to characterize the TMS-evoked muscle artifacts in TMS–EEG signal. For this purpose, three subjects underwent EEG measurement while they were given TMS pulses with various stimulation parameters. We systematically mapped the muscle-artifact contamination in different locations across the head to study which stimulation targets are likely to result in significant muscle-artifact contamination. The obtained muscle-artifact maps are shown in Fig. 5.1. We also visualized the spatiotemporal features of typical muscle artifacts to help researchers to identify muscular contamination correctly when analyzing TMS–EEG data.

In addition, we studied how different stimulation parameters, *i.e.*, the orientation of the primary induced electric field, the tilt of the coil with respect to the surface of the head, and stimulation intensity, affect the amplitude of muscle artifacts. Following the obtained results, we recommend to apply TMS mainly to central cortical areas. If answering the research question requires stimulating a more lateral area, then it can be beneficial to decrease the stimulation intensity. When possible, those coil orientations and tilts that maximize the distance between the coil windings and the scalp muscles should be favored.
5.2 Publication II: TMS-evoked changes in brain-state dynamics quantified by using EEG data

We studied whether TMS-elicited changes in the cortical dynamics could be quantified from trial-level TMS–EEG data. Often in TMS–EEG literature, only the average EEG responses to TMS are considered. However, the cortical activity followed by TMS can be inherently complex, varying considerably across the trials. Thus, by averaging we might lose a lot of information of the effects of TMS.

The hypothesis was that in the absence of external stimuli, the spontaneous EEG reflects the natural, idle brain state. When a TMS-pulse is delivered, the brain is shifted to a less probable state. For the period when the brain is disturbed from its natural state, the EEG activity is enhanced until the brain has recovered to the normal fluctuation level. The hypothesis is visualized in Fig. 5.2.

The immediate effects of TMS were quantified with two measures: the mean state shift (MSS) and the state variance (SV). MSS quantifies the
immediate TMS-elicited shift in the brain state, and SV measures the overall fluctuation in the primary currents. A significant increase in both MSS and SV support the hypothesis of the effects of TMS.

Figure 5.2. The hypothesis of the effects of TMS on cortical activity. A: TMS suddenly generates post-synaptic currents in a focal region. B: This change in the brain activity can be seen as a rapid shift in the brain state, which ultimately causes enhanced fluctuation in the brain activity until the brain has returned to the natural state. EEG measures a projection of the brain state and could potentially hold information of the rapid changes in the brain-state dynamics.

5.3 Publication III: Recovering TMS-evoked EEG responses masked by muscle artifacts

It is not always possible to follow the measurement recommendation suggested in Publication I, and thus, muscle artifacts may appear in TMS–EEG data. Therefore, we developed SSP methodology to suppress muscle artifacts. Mäki and Ilmoniemi had previously shown that to reject muscle-artifact dimensions, an appropriate SSP operator can be estimated from high-pass filtered data [134].

The main problem with the plain SSP approach is that the cleaned EEG signals can be heavily distorted. Partially, the problem is due to the misleading visualization. After SSP, the remaining, approximately artifact-free, virtual channels do not correspond to any of the original physical EEG channels. Thus, the traditional visualization of EEG traces and topographies after SSP can be misleading. We tested whether MNE extrapolation could be used to recover the traces in the original EEG channels. We developed an enhanced SSP method that was called the com-
combined signal-space projection and source-informed reconstruction method (SSP–SIR). The principle of SSP–SIR is illustrated in Fig. 5.3.

SSP–SIR was tested by applying it to three TMS–EEG datasets where TMS was targeted to M1. In addition, a simulation analysis was carried out showing that SSP–SIR conserves especially well EEG signals generated by those cortical current sources that are either tangential to the scalp surface or that are far enough from the scalp muscles.

**Figure 5.3.** Cleaning muscle-artifact contaminated TMS–EEG data with SSP–SIR. Starting from the upper left corner, the figure illustrates TMS-evoked EEG data at different stages of the developed cleaning method.
5.4 Publication IV: Automatic and robust noise suppression in EEG and MEG: The SOUND algorithm

EEG sensors are susceptible to several noise and artifact sources. This is especially true when EEG is combined with simultaneous electromagnetic brain stimulation. To suppress measurement noise and artifacts, we developed a method called source-estimate-utilizing noise-discarding algorithm (SOUND). SOUND is based on cross-validation and Wiener filtering. The measurement in a specific channel is compared to an estimate that is obtained from all the other sensors by means of MNE extrapolation (Eq. (4.5)). This procedure is repeated in an interactive manner until a reliable noise estimate in each sensor is obtained (See Fig. 5.4 for visual clarification). The final noise estimates are used for regularizing MNE. From the resulting, minimal-noise current estimates, the noise-suppressed versions of the sensor signals can be computed. With certain assumptions, the final noise-suppressed signals correspond to Wiener estimates (Eq. (4.13)).

SOUND was verified to work with real EEG and MEG data. The simulations showed that SOUND was robust against small modelling errors. Often in the EEG literature, measurement noise is tackled by visually inspecting the data, and then rejecting the contaminated sensors. The rejected sensor signals can then be interpolated, e.g., with spline interpolation [174, 180, 140]. In the simulations, SOUND produced better results compared to the sensor-rejection-and-interpolation scheme. Furthermore, SOUND cleans sensor signals automatically with very little user input. In the article, we also presented a general, data-driven Wiener approach that can be used to any dimension of data. As a proof of concept, we illustrated how the data-driven Wiener estimation can be utilized to take into account noisy trials when estimating the evoked EEG responses.
Summary of Publications

Figure 5.4. SOUND evaluates the noise levels in each channel by cross-validating the sensor signals via MNE extrapolation. The noise is estimated as the difference between the estimated and the measured signals in the circled channel. The obtained noise amplitude is stored in the noise covariance matrix, $\hat{\Sigma}$, which can be used in the later iteration steps to enhance the estimation (MNE extrapolation) accuracy.

5.5 Publication V: Signal-space projection suppresses the tACS artifact in EEG recordings

We studied the removal of tACS-induced EEG artifact. So far, the modulating effects of tACS on brain oscillation have been demonstrated mostly indirectly. For instance, changes in performing cognitive tasks during tACS have been interpreted as indications of shifts in the cortical state [183]. Another approach has been to compare the frequency content of EEG signals measured before and after the stimulation [252, 240, 148]. To gain more profound understanding on the cortical effects of tACS, it is of interest to measure EEG during stimulation. Because the brain activity during tACS is unknown, it is difficult to judge directly from human tACS–EEG data whether the applied artifact-rejection techniques have been successful.

To circumvent this problem, we built a tACS–EEG phantom (See Fig. 5.5) that captured the most essential physical aspects of the human head. In the phantom, SSP was able to recover the underlying oscillatory activity during tACS, demonstrating that SSP can be effective in suppressing the tACS artifact. We further validated the method in a human study,
where we were able to uncover the visual-stimulus-evoked event-related synchronization at alpha frequency, under the tACS artifact at the same frequency band.

Figure 5.5. In Publication V, the removal of the tACS–EEG artifact was studied in a phantom head. The phantom consisted of three layers mimicking the conductive properties of brain, skull, and scalp, and an internal dipolar neural source reflecting oscillatory activity. During an EEG measurement, a sinusoidal current was driven through the phantom to model tACS. A: Schematic illustration of the signal-delivery and recording setup in the phantom. Arrowheads indicate the direction of the information flow. B: Photo of the phantom head. C: Cross section of the phantom head along the midline. D: Stereographic projection of the phantom from above, depicting the locations of EEG (circles) and tACS electrodes (blue and red squares), and the dipolar source.
Combining electromagnetic brain stimulation with concurrent EEG can help us to gain better understanding of the functional roles of different brain regions and rhythms. However, the use of TEBS–EEG has been highly restricted to specific research questions because of several technical challenges that have not been overcome. In this Thesis, we showed how many of these problems can be successfully tackled.

TMS–EEG has great potential for measuring effective connectivity. Since TMS can be targeted anywhere on the cortex, the projections from, in principle, any ROI could be mapped with TMS–EEG. In practice, such a flexible use of TMS–EEG has not been possible because of the large muscle artifacts, which are easily generated when aiming TMS to lateral areas. Indeed, most of the TMS–EEG papers concentrate on studying brain regions close to the vertex of the head.

In Publication I, we mapped systematically how the level of muscle-artifact contamination depends on the TMS parameters, i.e., the stimulus location, (See Fig. 5.1), the coil orientation with respect to the head, and the stimulation intensity. Moreover, we characterized the spatiotemporal features of the muscular artifacts. The published results have already helped in planning TMS–EEG measurements [254, 28, 153] as we all as to correctly identify muscle artifacts when using ICA to remove these disturbances [185]. Obtained results were consistent with previous descriptions of muscle artifacts [118] and have been subsequently replicated [195].

Although, in Publication I, we showed that the muscle-artifact contamination can be decreased by altering the coil orientation or decreasing the stimulation intensity, a more thorough investigation of the tradeoff between the neuronal and muscular signal amplitudes as a function of the
stimulation parameters is needed. Indeed, it has been shown that also the neuronal EEG responses are sensitive to changes in these parameters [112, 29]. In addition, it would be useful to quantify what is the exact relevance of the capacitive properties of the skin–electrode interface [100] in the observed early artifacts. This information could help us to model the muscle responses more accurately, allowing an enhanced artifact rejection.

Research questions largely determine the appropriate stimulation parameters, and often muscle artifacts cannot be completely avoided. In Publication III, we introduced the SSP–SIR method, which suppresses the muscle artifacts while retaining the topographies of the neuronal EEG signals. We showed that SSP–SIR is able to preserve also the early TMS-evoked responses that overlap in time with the artifact. This improves greatly the reliability of effective-connectivity and cortical-reactivity studies where it is essential that also the early neuronal deflections can be observed. For instance, ICA has been shown to remove the muscle artifacts [118, 85, 196], but the resulting EEG traces during the first few tens of milliseconds tend to show low signal power, indicating that the early neuronal EEG responses might be compromised in the cleaning process.

Because SSP–SIR is computationally inexpensive, it suits well for cleaning large numbers of datasets or for processing data online, e.g., in the case of closed-loop TMS–EEG. Compared to ICA methods [118, 85, 196], the use of SSP–SIR requires little heuristic knowledge from the user. We showed that SSP–SIR works well when targeting M1. The use of the method should be further analyzed when targeting more challenging parts of the brain (See Fig. 5.1). The simulations in Publication III indicate that especially the signals generated by the neuronal sources right under the TMS coil can be attenuated by SSP–SIR. It should be noted that SSP–SIR does not provide more neuronal information compared to the previously presented plain SSP approach [134]. However, SSP–SIR does result in EEG signals that are easier to interpret because the data are reconstructed in the original EEG system.

Especially during TEBS, EEG data often suffer from a number of disturbances that are manifested as bad-quality channels and trials. In Publication IV, we showed how noise can be identified and cleaned automatically using Wiener filtering. In particular, we introduced the SOUND algo-
rithm, which was very effective in suppressing noise and artifact signals both in conventional EEG, as well as in TMS–EEG data. SOUND can be utilized also with other linear measurements, such as MEG [73], as long as the signals of interest in different sensors are not perfectly linearly independent and the appropriate forward model is known with sufficient accuracy. SOUND is both less time-consuming and utilizes the gathered data more optimally than the current EEG golden standard, channel-rejection and interpolation scheme [101, 27, 7]. SOUND was also shown to be much more robust compared to a previously presented Wiener-filtering based EEG/MEG cleaning approach, sensor noise suppression [44].

Today, there exists a number of recommended EEG-preprocessing pipelines [18, 98, 159] that attempt to remove noise components from the analyzed data. Comparing SOUND directly to these full pipelines is difficult because SOUND is only designed for a specific signal-processing step. Nonetheless, many of the presented preprocessing pipelines partially rely on the conventional rejection and interpolation of bad channels [18, 98], which was shown to perform worse than SOUND, in the simulations.

In Publication IV, we made some simplifying assumptions: the measurement noise was assumed uncorrelated across the channels whereas the primary currents were assumed independent and identically distributed. In the future, we should seek ways to estimate reliably the noise correlation matrix while allowing correlated noise structure. The presented version of SOUND is meant to reject only noise sources of extracranial origin. However, if we have good prior knowledge of the cortical activity in ROI, we can already incorporate this information into SOUND in terms of the primary-current correlation matrix, enhancing the performance of SOUND to reject neural noise.

Based on the work presented in this Thesis, the following protocol to obtain good quality TMS–EEG data is suggested:

1. Plan the measurements carefully; within the limits of the research question, choose the stimulation parameters so that the muscle artifacts are minimized.

2. In the post signal-processing, use SOUND to detect and correct contaminated EEG channels.

3. Finally, use SSP–SIR to clean any remaining muscle artifacts.
In TMS–EEG literature, the common approach to study the cortical effects of TMS is to analyze the average response. While this is a valid approach for many research questions, it fails to quantify the immediate changes in the ongoing cortical activity. In Publication II, we showed an alternative approach to frequency-domain measures [198] for quantifying the transient effects of TMS on a single-trial level. The presented measures and concepts might be useful in the future, e.g., in the closed-loop stimulation studies where it is essential to measure changes in the neuronal activity, online. Publication II can be considered as an introduction of a framework where, instead of observing the potential differences measured by single channels, we interpret EEG as a direct projection of the primary currents. This approach can help us to understand the effects of TMS on the brain state and how it compares with the natural activation mechanisms. In Publication II, we concentrated on studying the differences between the spontaneous EEG and the post TMS signals. Thus, we do not know how the observed TMS-elicited cortical-activity changes depend on the stimulated area or the overall state of the brain. One difficulty in quantifying the immediate TMS-elicited effects is the inherently low SNR in the single-trial level data. This problem could be partially solved by applying methods such as SOUND prior to quantification.

In Publication V, we tackled the tACS-induced EEG artifact. The results can be considered promising, since in the phantom studies the tACS artifact seemed to be completely rejected while the underlying neuronal EEG was retained well. There have been earlier approaches to clean tACS-related artifacts from EEG signals [80, 239] but this is the first time when a phantom was used to verify that the remaining data reflects genuine signals of interest and not just a residual artifact. Further human studies supported the phantom results.

However, the tACS–EEG study has some limitations that need to be addressed. In the phantom, the neuronal oscillation was not phase coupled with the tACS artifact. Because of the way the SSP operator was estimated from the average tACS-artifact template, it is likely that at least parts of the EEG signals reflecting the entrained oscillations at the stimulation frequency would be attenuated by the artifact-rejection method. In the future, it would be of particular interest to perform a study where the tACS-currents would consist of two or more frequencies and amplitudes.
In such a case, the spatial filter would not have to be estimated directly from the frequencies of interest.

It has been suggested that instead of EEG, concurrent MEG could be used to measure the immediate effects of tACS [212, 150]. Whether this is a reliable approach is still a matter of debate [160, 132]. A credible rejection of the tACS artifact is extremely important because it enables to verify directly the possible entrainment effects. However, the true impact of the methods developed in Publication V depends largely on the significance of the online effects of tACS, which have not been yet reliably demonstrated [160, 132, 121].

It is important to note that the presented SSP-based methods and SOUND are all based on spatial modelling of the noise and artifact signals. Thus, these methods might not function ideally with sparse EEG-sensor layouts. However, when using high-density EEG-sensor systems, they offer a potential alternative for rejecting disturbances that, either, overlap heavily in the frequency domain with the signals of interest, or are otherwise difficult to disentangle in the time domain.
Discussion
7. Conclusion

In the publications included in this Thesis, we have introduced a number of methodological solutions to some of the most cumbersome technical challenges related with combining TMS or tACS with simultaneous EEG measurement. The presented work provides guidelines for recording cleaner signals. Furthermore, several signal-processing methods, for rejecting unavoidable noise and artifact signals, were developed. Finally, novel measures for quantifying the TMS-induced effects on the ongoing cortical activity were introduced.

The new tools help to prevent unnecessary loss of data and information, decreasing the time and effort in the data acquisition. Similarly, because the presented noise- and artifact-cleaning tools require relatively little heuristic knowledge, their use simplifies the signal-processing stage, potentially resulting in more reproducible results.

The obtained results enable a reliable combination of TEBS with concurrent EEG in many of today's brain-stimulation applications and can help in developing novel study paradigms, such as closed-loop stimulation. The taken steps are also essential for bringing TEBS–EEG closer to clinical applications. To summarize, this Thesis presents novel experimental and signal-analysis methodology that allows us to uncover and quantify TEBS-evoked neuronal EEG signals in new, previously infeasible, study designs.
Conclusion
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