Closed-loop transcranial electrical stimulation: novel techniques for integrating electroencephalography and real-time adjustments of a.c. stimulation

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Abstract

The goal of this thesis is to design methods and techniques for closed-loop transcranial Electric Stimulation (tES) based on feedback from ongoing neural activity. tES is a popular Non-Invasive Brain Stimulation (NIBS) approach and has been shown to modulate ongoing brain activity to affect behaviour. The application of these methods has major implications in both understanding the anatomy & function of the brain, and development of therapies for non-pharmacological intervention in mental health disorders. A review of existing brain imaging techniques compatible with tES shows that electroencephalography (EEG) is the most suitable method to pair with tES to design the closed-loop stimulation interface. Furthermore, of the different forms of tES, transcranial Alternating Current Stimulation (tACS) is the ideal method to use for closed-loop stimulation due to its ability to modulate ongoing neural activity in a phase specific manner.

However, most studies have been limited to exploring changes in EEG before and after stimulation due to the presence of stimulation artifacts in the EEG data. A characterisation of these artifacts and a review of existing methods will identify the shortcomings of current practices. Thus, two novel algorithms for tACS artifact removal are be presented. Further, methods to judge the performance of such algorithms are currently limited and thus, new techniques to comprehensively test and verify these algorithms are presented, including the use of a novel phantom head model.

A proof of concept for assessment of EEG activity during tACS is presented using novel methods that allow monitoring working memory during stimulation, via successful classification of data during two different tasks. This presents a novel technique to both verify artifact removal and also monitor ongoing neural activity during stimulation. Finally, an interface that executes the developed techniques in real-time was built. This toolbox is subsequently capable of providing closed-loop feedback to adjust tACS parameters based on ongoing EEG activity. It was designed to be as independent of hardware as possible, making it easy for other researchers to employ this toolbox in different labs across the world. This will allow for easier repeatability of methods in the field of tES research, which is a known issue.

In summary, the novel techniques presented in this thesis are key steps towards development of closed-loop tACS as a tool for personalised, non-pharmacological therapy in mental health disorders.
Declaration of originality

I hereby confirm that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.
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Chapter 1

Introduction

1.1 Thesis Overview

In recent years there has been considerable development and growth of non-invasive brain stimulation technologies. Amongst them, transcranial Electrical Stimulation (tES), is a relatively new technique with many potential applications as a therapeutic tool [1]. tES can be thought of as a non-invasive analogue of Deep Brain Stimulation (DBS), where electrodes are surgically implanted into the brain. These electrodes stimulate currents of 0.3-3.5 mA [2]. This technique is applied in treatment of Parkinson’s disease [2] and epilepsy [3]. However, due to its invasive nature, the application of this technique is very complicated and carries multiple risks such as surgical complications (haemorrhage and infection) and the efficacy reduces over time [4].

Thus, recent years have seen a growing trend in the development of non-invasive brain stimulation technologies. Of these transcranial stimulation techniques, Transcranial Magnetic Stimulation (TMS) [5] is the best known and widely used. It operates by using a large magnetic field applied outside of the head to induce small currents within the head. TMS has been applied to affect motor, visual, and auditory function as well as cognitive learning. Its use in the clinic and its ability to modulate cortical activity have been extensively explored [5]. However, TMS equipment is expensive, bulky and requires considerable training to operate.

As a result, tES has been considerably developed in recent years, gaining increasing popularity both in research literature and as a therapeutic tool. tES operates by injecting small amounts of current into the scalp via rubber electrodes that are enclosed in saline soaked sponges and placed on the scalp (See Fig. 1.1). It is available in several forms: transcranial Direct Current Stimulation (tDCS) applies constant current and is the best known; transcranial Alternating Current Stimulation (tACS) applies sinusoidal alternating currents and is also widely used; random noise stimulation (tRNS) and pulsed current stimulation (tPCS) forms are also available [6]. tES devices are cheap, portable, non-invasive and easy to use, thus providing new approaches for therapeutic interventions as an alternative to the expensive and risky procedure such as DBS.
Of the different forms of tES, transcranial Alternating Current Stimulation (tACS) was identified as the focus of this thesis. There are multiple frequencies that neural networks oscillate at, while performing various different tasks and the injection of currents at these certain frequencies provide a platform to directly modulate the ongoing processes. Although, various studies have presented the unique potential of tACS as a tool to modulate ongoing oscillatory activity in the brain (discussed in Section 2.4), there remains little understanding of the mechanisms behind its effects. Existing methods did not allow simultaneous brain monitoring to be performed, allowing the effects of the tACS to be observed during stimulation. This is essential to understanding the operation of tACS and proving its utility, moreover if this could be done in real-time, it would be possible to dynamically adjust the stimulation parameters on an individual by individual basis, which has previously never been possible.

The goal of this thesis is to integrate tACS with simultaneous brain monitoring via the electroencephalogram (EEG). This is done in both offline and online formats providing new tools for performing tACS experiments. The key challenge in this is the tACS stimulation artifact that occurs in simultaneous EEG measurements and creating suitable signal processing for removing this.

To achieve this the following targets were identified:

- To create algorithms for removing tACS stimulation artifact from simultaneous EEG recordings;
• To verify and validate the performance of this artifact removal, requiring methodo-
logical advancement of the existing performance assessments;

• Algorithms to monitor and evaluate brain activity during stimulation;

• To create artifact removal methods which have sufficiently low computational com-
plexity to run in real-time;

• Use of monitoring algorithms during real-time artifact removal to demonstrate the
first closed loop EEG-tACS system where some stimulation parameters can be dy-
amically changed to match the underlying EEG during stimulation.

1.2 Thesis Structure

Following a thorough review of existing work, I identified key technological milestones
that are required to achieve closed-loop functionality in tACS protocols. The first part
of this thesis will focus on critical designing of techniques for removal of the tACS ar-
tifact from EEG data collected during stimulation. Subsequently, I establish a proof of
concept for online assessment of working memory during stimulation which allows for
future work to build upon presented results and create closed-loop protocols for work-
ing memory enhancement in mental health. Finally, I present a platform in the form of a
toolbox that operates artifact removal and online assessment in real-time and, is capable
of closed-loop operation. This will allow other researchers to easily repeat these tech-
niques and build upon them in the future.

1.2.1 Chapter 2 - Overview of neuroimaging and elecrical stimulation

In this chapter, I will provide a brief overview of some critical concepts necessary when
designing any techniques using electrical stimulation and neuroimaging. A basic under-
standing of how the both neuroimaging captures the function of the brain and electrical
stimulation modulates it will be presented.

After reviewing the various functional neuroimaging techniques that are compatible with
tES, I will present why the use of EEG to monitor brain activity with concurrent elec-
trical stimulation is the best choice. Similar evaluation of the different methods of tES
available will also be discussed and thereby providing the rationale behind choosing
tACS as the focus of this study. The complimentary nature of EEG and tECS will be
presented with the goal to target the potential applications of tACS using closed-loop
operation. Also a review of the safety and stimulation guidelines that was taken into ac-
count when designing the stimulation protocols for this study and, applying for the rele-
vant ethics approval, will also be presented.
However it should be noted that more specific, relevant literature reviews will be presented at the start of each subsequent chapter to present further context and motivation of the presented content.

1.2.2 Chapter 3 - Designing techniques for tACS-EEG Artifact Removal

In this chapter, I will begin by providing the basic requirements to set up compatible tACS-EEG montages. I will present characterisation of the tACS artifact on EEG data and subsequently review existing techniques for artifact removal. On the basis of this review, I present key features that I incorporated while designing the presented algorithms used in this study. Two novel techniques for tACS-EEG artifact removal will be presented.

In summary, I will have presented two new methods for tACS-EEG artifact removal that are compatible for real-time operation and are channel independent making them ideal for applications for closed-loop techniques.

1.2.3 Chapter 4 - Testing and verification of tACS-EEG Artifact Removal

A novel phantom head model developed to test and characterise the performance of the new methods for artifact removal is presented in Chapter 4. Furthermore, this phantom head model will allow a method for testing that is easily repeatable and also a platform to compare the presented and future techniques.

After testing using the phantom head model, the next step is to verify the performance of the novel techniques presented in chapter 3 using on-subject data. Furthermore, a review of presented techniques in the field shows a lack of comparison for different methods and subsequently a rush to present behavioural results using these methods. A major reason for the missing steps of evaluation and comparison of techniques is the lack of established methods to do so. Further, establishing these methods is not easy due to the nature of the problem.

Thus, I will present a study that compares the performance of the two new methods introduced in chapter 3 with an existing method. I present a multi-stage approach which looks at detecting alpha activity in the frequency domain and evoked responses in the time domain. As well as statistical comparison of descriptive statistics of the data. We use all these separate analysis to build confidence on the validity of reconstructed data after tACS artifact removal using the three different methods.
1.2.4 Chapter 5 - EEG monitoring of working memory during tACS

Once the performance of the artifact removal was verified, the next step in the development of the closed-loop operation was to establish the ability to assess data during stimulation and to provide feedback for any closed-loop operation. The use of tACS as an intervention for working memory enhancement is discussed and the great identified potential of future applications in MCI, Parkinson’s and Alzheimer’s and other neurodegenerative diseases motivates this chapter.

Therefore as a proof of concept, I designed a study where tACS was applied subjects while they performed two different working memory tasks. The goal of the study was not to present any behavioural effects of stimulation but rather present the ability to monitor working memory using EEG during tACS.

Data collected during the task, with (after artifact removal) and without stimulation was used to train a machine learning classifier using dynamic features based on discrete wavelet transforms. Successful separation of the different tasks and baseline condition both with and without stimulation not only adds further verification of artifact removal but is also the first study to report the ability to monitor working memory during stimulation.

1.2.5 Chapter 6 - The tACS-EEG toolbox

The final stage of the development of the closed-loop techniques requires an interface with the ability to both control the EEG and tACS equipment and, assess the ongoing EEG to subsequently adjust stimulation parameters. Thus I designed the tACS-EEG toolbox as a plug-in for Matlab (Mathworks Inc.) which is capable of such operation.

The first stage was the adaptation of the artifact removal techniques to operate in real-time. Subsequently, an EEG stream of data was established using two different EEG devices and a system to control the stimulator using a Data Acquisition Card (DAQ). Cognitive tasks and the subsequent online assessment is also included for the control of the closed-loop operation. Performance of the toolbox is tested using the phantom head model presented in Chapter 4. Both the latency/throughput of the toolbox operation and the artifact removal performance in real time is evaluated.

The goal is to release the toolbox as an open source tool such that groups from across the world can use the methods and techniques designed over the course of this thesis. This common platform will allow researchers to test and compare existing and new methods to select the best setup for their studies and, it also provides a tool to document their protocols that can then easily be replicated in the future.
1.2.6 Chapter 7 - Conclusions and Future work

In chapter 7, I present an overall summary of the thesis. The techniques and methods introduced in this thesis are key steps towards the design and use of closed-loop methods using electrical stimulation. The created techniques for artifact removal are designed to be as independent of hardware as possible so that they are easily accessible at little to no extra cost to researchers. The methods for evaluation of these techniques are also novel and present not only a common tool for others to compare existing and new methods but also have further applications in the field. The presented work is the first to establish the monitoring of EEG activity using dynamic features during tACS and these methods can allow researchers to design protocols for the use of tACS as a therapeutic tool in various different mental health disorders. Finally this will lead to multiple avenues for future research projects that stem out of these findings and some of these avenues will be presented in this chapter.

1.2.7 Summary

In summary, I will have presented novel methods and techniques by the end of this thesis. Each of these are critical steps towards the design of simultaneous EEG monitoring during tACS and subsequently any closed-loop adjustment of the stimulation parameters. By the end, a novel interface will be presented that is capable of closed-loop tACS-EEG operation and has been tested using phantom heads and on-subject data. The next step is the continued development of this interface to design therapies in mental health.

References


Chapter 2

Overview of neuroimaging and electrical stimulation

2.1 Introduction

The integration of transcranial electrical Stimulation (tES) and Electroencephalography (EEG) is a multi-disciplinary topic combining concepts from neuroimaging, neuromodulation and signal processing fields. Thus, before presenting the novel techniques and methods, it is necessary to provide some of the basic principles behind the functionality of tES and the imaging techniques that are used in conjunction with it. However, this chapter does not provide a full review of all literature, specific literature discussions are provided for relevant literature in each individual chapter.

Section 2.2 of this chapter introduces a basic understanding of the different forms of neuroimaging that have been applied in conjunction with tES and thus, present the reasoning behind choosing EEG as the method to pair with tACS in this study. This is crucial when algorithms for artifact removal are designed and tested, as any successful technique will require an understanding of the operation of the respective imaging techniques.

Next, in Section 2.3, a brief introduction to tES will be presented in a summary introducing the stimulation and safety guidelines and the different forms of tES. Finally, in Section 2.4 the application of tACS in particular to modulate ongoing neural oscillations will be discussed, describing the motivation for developing methods that utilise online assessment of EEG activity during tACS to adjust stimulation parameters.

The understanding of the principles behind the imaging and stimulation techniques discussed in this chapter is prerequisite to the development of closed-loop operation of tACS.
2.2 Neuroimaging with tES

2.2.1 Introduction

There are many different methods available for the invasive and non-invasive monitoring of the brain. These methods provide different trade-offs between the temporal and spatial resolution, ease of use, portability, expense and other factors. The different modalities available are introduced here to motivate the choice of EEG neuroimaging in the remainder of this thesis and give background concepts core to the application of EEG.

I briefly present certain imaging techniques that researchers have used in combination with tES. I mainly focus on EEG since that is the technique employed for this study because, as will be explained below, it is the most compatible with tES for designing closed-loop stimulation protocols.

2.2.2 Magnetoencephalography (MEG)

Magnetoencephalography (MEG) detects weak magnetic fields induced by the currents generated during neural activity [1]. These currents are derived from the flow of ions during transmission of action potentials in dendrites and the resulting magnetic fields are very small, much smaller than background magnetic noises. Therefore, a cluster of neurons is required to be active for signals to be detected using MEG, giving it a spatial resolution of 4-8 cms. However, the time resolution of the MEG signals is very high, in the region of milliseconds, and this makes it a suitable imaging method for closed-loop operation since the rate of feedback to the interface directly influences the number of tasks per minute that can be achieved.

[2] presented the use of beamforming filters for artifact removal during tACS stimulation. They showed evoked responses to alpha activity onset and visual/audio stimuli, but a quantified evaluation of performance or comparison to other techniques was lacking. Subsequently, [3] also presented techniques for EEG recording during tACS but they present their results on a phantom head model and use amplitude modified tACS, the effect of which may be different from typical tACS waveforms, see Chapter 3 for detailed discussion.

The major limitations of MEG for use in this study are the lack of mobility of this technique and the cost of high quality devices. The weak signals are hard to detect and can be easily influenced by noise and thus, most MEG recorders either require subjects to be stationary and be in shielded rooms, or any available ‘mobile’ units are very bulky and remain very sensitive to noise. Furthermore, the running costs of MEG units can be considerable due to the high power consumption of units.
2.2.3 Functional Magnetic Resonance Imaging (fMRI)

Paul Lauterbur, [4], is credited with inventing Magnetic Resonance Imaging (MRI) in 1973 and by 1981, the first images of the human brain were obtained [5] and the use of MRI to detect tumors, stroke and multiple sclerosis was established [6],[7].

Simply stating, an MRI works by applying a very strong magnetic field that aligns a proton ‘spin’ and a radio frequency current is also produced by the scanner that causes hydrogen nuclei (of water molecules in human body) to ‘flip their spin’. When the field is turned off, the nuclei return to their normal spin. This is called the precession and, receivers in the scanner can detect this precession to create an image [4]. Subsequently in 1990, Ogawa et al. [8] were amongst the first to conceptualise the Blood-to-Oxygen Level Dependant (BOLD) contrast imaging. This relied on the fact that the activation of neurons required energy which in turn would require different levels of oxyhemoglobin and deoxyhemoglobin, which have different magnetic properties that can be detected and thus, the technique of functional-MRI (fMRI) was established. The ability to detect oxygen consumption allows the detection of active/inactive brain regions during tasks, providing crucial information on the functionality of the human brain.

Previous studies have included the use of fMRI to inform tES dynamics. [9] explored the effect of alpha tACS on the BOLD signal and found no direct BOLD signals as a result of stimulation, but rather a change in BOLD activity due to visual task after stimulation. The BOLD signal changes due to visual stimuli were both reduced and increased due to tACS at the individual alpha frequency (IAF) and delta (1 Hz). These results along with the lack of effect at resting states suggest a state-dependant efficacy of tACS. Furthermore, [10], attempted to classify the imaging artifacts of tES using fMRI by stimulating on post-mortem subjects. They found tDCS caused induced signals on both surface and deep structures, whereas tACS did not induce any signals that were detected by the fMRI, correlating with [9]’s findings that tACS effects ongoing processes but does not directly cause BOLD activity. Recently, [11] recorded fMRI activity during an intelligence task post theta tACS at the left parietal region. They present a decrease in activity post stimulation in regions that are not occupied during the task, and also a lowered activation further adding to the task-specific efficacy of tACS and suggesting that stimulation increases neural efficiency.

A recent study, [12], presents a unique approach for combining tACS and fMRI. Real-time fMRI during tACS was used to obtain current brain state images which were compared with target brain states and, subsequently using machine learning and bayesian optimisation [13] the tACS parameters (phase and frequency) were adjusted for the next block of stimulation. Although this is a form of ‘closed-loop’ stimulation, but it is not the same operation which is the goal of this study, i.e., here the closed-loop operation is optimising stimulation to achieve a pre-determined brain state. Methods such as this
allow researchers to broaden the scope of hypothesis testing by ‘supervised machine learning’, however, this method still has the limit of applying closed-loop optimisation to achieve predetermined target brain states but not interact with ongoing neural activity to achieve desired behavioural effects, which is the goal of this study.

In summary, imaging using fMRI has the benefits of very high spatial resolution (millimetres) and no depth sensitivity limitations however, it is not suitable for closed-loop functionality with out-of-lab applications due to its low temporal resolution (1-10s), lack of mobility and high costs.

**2.2.4 Electroencephalography (EEG)**

EEG is a non-invasive technique used to record brain activity. It uses small electrodes placed on the scalp which measure changes in the electrical activity resulting from the activity of neurons in the brain. Each channel consists of an amplifier that measures the difference in electrical potential between a recording electrode and a reference electrode, see Fig.2.1. This recorded difference is indirectly the result of changes in voltage potentials at the site of the electrodes caused by the ionic flow during action potentials [14]. The recorded activity is usually around 0 − 100µV peak-to-peak for adults, and spread across a bandwidth of 0−100Hz, [15]. The electrodes are placed on the scalp using standard configurations developed to target specific locations, the international 10-20 system is displayed in Fig. 2.2.

EEG has a similar time resolution to MEG units (ms) and has a slightly higher spatial resolution (approx. 10 cm). Also recently, much smaller EEG units have been made available, thus making them very suitable for operation in mobile, out-of-lab settings. Furthermore, due to the decrease in cost and size of transistors, as well as developments in wireless and bluetooth technologies, multiple low-cost wireless EEG units are now easily available, some even targeted for commercial use.

Fig. 2.3 shows some of the available mobile EEG units. The products targeted for commercial use usually vary in cost from £100-1k whereas, the research grade units cost on average from £5-10k. There has been a real push in recent years to move the use of EEG towards out-of-lab applications, and thus there is an increasing amount of research looking into collecting data in mobile environments. This research is mainly focused on

![Figure 2.1: An EEG amplifier](image)

```python
Rec Channel

Ref Channel

EEG
```
two fronts, the development of algorithms using machine learning for real-time analysis and also the design of dry electrodes for easier and quicker setups with low sensitivity to noise. A study published in 2014, [16], presents the use of a visual P300 spelling task using a standard EEG amplifier and a mobile EEG amplifier (Emotive adapted to use with wet electrodes). Their results show both similar performances of the two devices and P300 amplitudes, showing that mobile EEG units can perform as well as existing lab amplifiers however, they used the mobile unit in lab conditions with wet electrodes. Thus there were minimal artifacts present and even with the comparable performances, there is still a step lacking in terms of out-of-lab applications both in terms of dealing with noise in real world environments, and quick and easy setup using dry electrodes. A study in 2016, [17], presented novel 3-D printed dry electrodes that can be manufactured using off-the-shelf components. A thorough characterisation shows that even though there is higher contact noise and drift, the presented electrodes are able to capture ongoing alpha oscillations albeit with a lower signal-to-noise ratio (i.e. more noise). Even with the lowered performance, the ability to 3-D print allows for quick manufacturing of electrodes which can potentially be customised to experiments individually, and are still able to capture evoked responses allowing for use in out-of-lab conditions with minimal setup time and no prior training required. A 2017 review, [18], of methods for out-of-lab EEG data acquisition in daily life show that though research exists for noise artifact removal during daily life tasks using mobile EEG devices, none of these studies use dry electrodes or collect data outside labs. They conclude that one of the main reasons for this is the lack of standard noise removal algorithms available, though recent work using methods such as source decomposition and wavelet transforms have shown promising

Figure 2.2: Left: A typical EEG set-up, Right: 10-20 system for electrode montage

25
Figure 2.3: Some Available mobile, wireless EEG units. Top row: Commercial units, Bottom row: Research grade units

Table 2.1: Frequency bands of EEG activity in brain and some common functions associated with the respective frequencies

<table>
<thead>
<tr>
<th>State</th>
<th>Frequency</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta</td>
<td>0.1-4 Hz</td>
<td>Slow sleep wave</td>
</tr>
<tr>
<td>Theta</td>
<td>4-8 Hz</td>
<td>Memory and Cognition</td>
</tr>
<tr>
<td>Alpha</td>
<td>8-12 Hz</td>
<td>Awake, Resting</td>
</tr>
<tr>
<td>Beta</td>
<td>12-30 Hz</td>
<td>Voluntary Motor action</td>
</tr>
<tr>
<td>Gamma</td>
<td>25-100 Hz</td>
<td>Visual binding and attention</td>
</tr>
</tbody>
</table>

Oscillations in the EEG in relation to a stimulation can be spontaneous (background activity), evoked (after stimulus and phase locked with stimulus) or induced (after stimulus but not phase locked) [19]. These oscillations (Table 2.1) contain important information which can be linked to various factors like cognition, memory and performance [20], [21]. For example: alpha frequencies (around 10 Hz) indicate the brain is in a resting state [22]; beta frequencies (12 to 25 Hz) are associated with motor actions such as voluntary movement [23]. Evoked and induced oscillations from gamma frequency stimulations have been found and proposed to be responsible for functions such as visual binding [24] and attention [25]. Changes in the oscillations can be related to different neurological disease states. In Alzheimer’s and Parkinson’s Disease the alpha rhythms and beta rhythms are affected respectively [26], [27]. In addition, the presence or absence of frequencies can be used to control computers through Brain-Computer Interfaces (BCIs)
Fig. 2.4 presents EEG activity in both time and frequency domain. Free running EEG data in the time domain for a single channel is shown along with the average Event Related Potentials (ERPs) for two different visual stimuli, face and non-face (pixelated scramble of respective face image). As seen, the average ERP for the face (black) has a higher amplitude after 170 ms (called the N170) post stimuli (i.e. after the image is shown) compared to the non-face stimuli (red). Also the Power Spectral Density (PSD) of a basic alpha induction task is shown. The induced alpha activity (i.e. activity at 8-12 Hz) when eyes are closed (blue) is higher than alpha activity when eyes are open (red), since the brain is in a resting state when eyes are closed. These evoked and induced features are used later as features to identify after artifact removal of tACS from EEG data, see Experimental Protocols in Section 4.3.2 for more details.

There is only one previous study that presents the removal of tACS artifacts from EEG data [29]. They apply Principle Component Analysis (PCA) to remove the tACS artifact, however this method is limited to EEG devices with high channel counts and is not compatible for real time operation. This method will be discussed in detail in Chapter 3, however it is clear that for closed-loop operation, combining tACS and EEG requires new algorithms for artifact removal.
2.2.5 Selecting neuroimaging method for tACS based BCI

A Brain-Computer-Interface (BCI) operates via direct communication with ongoing brain activity, traditional BCIs detect evoked responses in either the frequency and time domain to trigger commands to computers. For example, one of the most common EEG based BCIs is the P3000 speller used by the study discussed in section 2.2.4 [16], it uses the amplitude of a visual ERP 300 ms post stimuli (hence P300) to allow the user to select letters presented to them on a screen. This P300 speller is commonly used to allow paralysed patients to communicate.

Any experimental interface that will perform closed-loop tACS based on ongoing neural activity is classified as a BCI. Table 2.2 presents a summary of some key parameters of the three methods of neuroimaging discussed in this section. As seen, EEG and MEG have superior time resolution dynamics and benefit from directly recording electrical activity of the brain. MEG records activity resulting directly from action potentials and thus, records primary currents whereas EEG records the change in activity with regards to a reference electrode, i.e. secondary currents. MRI based techniques use consumption of oxygen to detect functional activity and have much lower time resolutions. These units are also very expensive and are limited to use in laboratory conditions. Although MEG, like EEG, also measures direct changes in ionic potential it remains, very expensive to own-and-operate mobile MEG units that are available, which still remain very bulky and sensitive to noise, and thus are not ideal to incorporate into the lives for potentially vulnerable patients.

<table>
<thead>
<tr>
<th></th>
<th>EEG</th>
<th>MEG</th>
<th>fMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal Type</td>
<td>Secondary Currents</td>
<td>Primary Currents</td>
<td>Indirect (BOLD)</td>
</tr>
<tr>
<td>Temporal Resolution</td>
<td>~ 1 ms</td>
<td>~ 1 ms</td>
<td>1 - 10 s</td>
</tr>
<tr>
<td>Spatial Resolution</td>
<td>~ 10 cm</td>
<td>~ 4 cm</td>
<td>~ 1 mm</td>
</tr>
<tr>
<td>Depth</td>
<td>~ 8 cm</td>
<td>~ 4 cm</td>
<td>Indifferent</td>
</tr>
<tr>
<td>Direction</td>
<td>Tangential and Radial</td>
<td>Tangential Only</td>
<td>Indifferent</td>
</tr>
</tbody>
</table>

Table 2.2: Comparison of EEG, MEG and fMRI

It was decided to incorporate EEG and tES techniques to design BCIs for this study. Both methods operate by placing electrodes on the scalp and either record or modulate the electrical potential at the electrode sites. They have high temporal resolutions allowing the easy modulation of one using feedback from the other.

2.2.6 Noise sources in EEG

Now that a suitable imaging method has been selected to pair with tACS, it is important to note other different sources of noise observed in EEG data. These can originate from both the subject’s body and natural/artificial sources in the surroundings. This section briefly discusses these artifacts and their characteristics. Furthermore, examples of some
of these are also displayed in Fig. 2.5. These artifacts were extracted from EEG data for a single subject collected while performing the tasks described in Section 4.3.2.

Of the biological sources, the most common artifacts in EEG data is from eye blinks and other muscle movements. These present as high-amplitude, low-frequency artifacts which are more prevalent on frontal electrodes or other electrodes placed on regions with minimal hair [30]. Fig. 2.5.B,C,E show artifacts at the Fp2 channel (located at the forehead) from eye blinks, muscle movement (eyelid closing) and lateral eyeball movement (ocular movement) respectively. Artifacts of eye blinks are often many times greater than the normal EEG data and can thus be often seen in all channels, even ones at the back of the head [31]. Artifacts resulting from muscle movements can vary based on the duration of muscle contraction and the location of said muscle and the placed EEG electrodes. The resulting artifacts are a product of both the electric field generated by the muscle and the movement of electrodes and their contact leads. As is seen in Fig. 2.5.C, where the closing of the eye results in a small DC shift in the signal which is a result of a slight shift of the frontal electrode (Fp2) and a subsequent change in the impedance of the electrode in relation to the ground and reference electrodes [30].

Figure 2.5: Some different forms of EEG noise artifacts extracted from data collected for this study.

The cornea of the eye has a slightly different electrical potential than the back of the eye, this potential is also varying and thus the difference of said potentials can be monitored to observe eye movements, which is the principle behind Electro-oculogram (EOG) [32]. Artifacts of ocular movement can be observed in frontal electrodes and manifest with low frequency (<1 Hz) and very low amplitude such that it resembles an unstable baseline for the ongoing EEG activity [30]. This is observed in Fig. 2.5.E. However monitoring EOG is also used to detect certain brain states, in particular the classification of REM sleep, i.e. rapid eye movement sleep, is defined by the increase in eye movement activity paired with slow EEG oscillations during sleep.
The ongoing bodily processes such as respiration, heartbeat and blood-flow can also manifest as artifacts in EEG. These vary in characteristics but the rhythmic nature of these processes is captured in the artifacts as well. The cardiac artifact is present in 2 forms, the mechanical is the artifact from the contraction of blood vessels in the neck and is commonly seen in temporal electrodes [30]. The electrical cardiac artifact is observed on the scalp and appears similar to an ECG signal, forming the QRS complex. Similarly respiration artifacts are similar in characteristics to ocular artifacts but retain the breathing rate. These are more common during slow wave activity. An example of respiration artifacts is shown in Fig. 2.5.D, the artifacts are similar to the previously discussed ocular artifact and appear at a rate of approx. 18 breaths per minute which is within the average respiration rate for healthy adults.

The most common external artifact which appears in all EEG data is interference from the mains electricity which is observed as constant 50 Hz noise (60 Hz in USA) as this is the frequency at which electricity is supplied [31]. Fig. 2.5.A shows unfiltered raw EEG data, a constant high frequency interference is observed to be superimposed on this data, this is mains noise. Although there is always a certain degree of transmission line noise in all collected data, the best practice to minimise this noise is to properly ground all electrodes and thus the resulting noise is usually of a medium to low amplitude is easily filtered. Other external sources of noise include mobile phones, electrodes and electrode lead movements, sweat and even an effect of the magnetic field of the earth.

Majority of the described artifacts can be suppressed by the use of filters. The most dominant EEG artifacts such as mains noise and eye blinking/movement have frequencies above of below brain oscillations respectively. Thus these can be removed using digital filters [30]. However artifacts with very high amplitudes, many times greater than EEG, such as eye blinks are not sufficiently removed by just the use of filters and commonly removed by eye inspection [30]. There also exist methods for automated removal of these artifacts, for example: blink artifacts have been shown to be removed using Independent component analysis (ICA) [33] and blind source separation [34]; [35] presented the use of discrete wavelet transforms (DWTs) to remove ECG artifacts from EEG data; [36] present an evaluation of EOG artifact removal using different methods such as EOG subtraction, ICA, Principle component analysis (PCA) and Joint approximate diagonalisation of eigenmatrices (JADE) and [37] show the use of adaptive filters to remove EOG data as well based on EOG recordings.

2.2.7 Summary

All three described neuroimaging methods record functional activity and are used in BCIs. Studies have investigated using all three methods in conjunction with tACS. However, both fMRI and MEG are lab restricted methods due to their bulkiness and sensitivity to noise and are also very expensive. Thus, due to the complimentary nature of tACS
and EEG, and the mobility and low costs of EEG devices, it was decided that EEG will be the chosen method to design the closed-loop tACS BCI.

Also presented was some basic EEG activity in the time and frequency domain which are evoked and induced respectively. The first goal of the described BCI will be to detect such activity during stimulation which can thus be used to evaluate and adjust stimulation parameters moving forward.

2.3 transcranial Electrical Stimulation (tES)

2.3.1 Introduction

Brain Imaging techniques have been available for almost a century, but recently technology has been made available which allows for direct intervention of brain activity electrically. tES operates on the principle of directly affecting the electrical potential of the brain to increase or decrease the rate of firing of targeted cortical regions.

Furthermore, its high temporal resolution makes tES an exciting tool for combining with brain imaging techniques to provide a platform for the development of closed loop BCIs which stimulate the brain depending on the activity recorded in real-time. EEG records the change in electrical potentials of the brain and tES is used to directly modulate said potentials, making the two naturally compliment each other and be used together going forwards to develop these next generation of BCIs.

This section introduces tES. Following a brief description of how electrical signals are generated in the brain in Section 2.3.2. I will present the different forms of stimulation in Section 2.3.3 and applications of tACS in particular are discussed in detail in Section 2.3.4 to present the rationale for choosing to design the closed-loop stimulation using this form of tES. Safety guidelines for tES are introduced in Section 2.3.5 and stimulation guidelines in Section 2.3.6, followed by a brief summary in Section 2.3.7.

2.3.2 Some basic neuroscience

The Central Nervous System (CNS) is a complex framework consisting of the brain, the spinal cord and a network sensory receptors which transmit information to and from the brain via the spinal cord [38]. Here, I present a brief overview of the basic operation of neurons and synapses, in particular the mechanism behind the generation of electrical activity in the brain which is directly modulated when applying any form of electrical stimulation to the brain.

The neuron is the basic building block of the CNS, the human anatomy has up to a 100 billion neurons [39]. Neurons use synaptic connections to communicate. The said ‘synapse’
Figure 2.6: The Action Potential

is found at the endings of dendrites and axons of neurons. Synapses receive and transmit information, at the dendrites and axons respectively. A neuron transmits information by sending an electrical signal through the axon called the action potential, see Fig. 2.6. This signal is a result of a chemical reaction which is generated by flow of ions in and out of the cell body. The resting potential of the neuron is at $-70 \text{ mV}$, if this is increased to the threshold of approx. $-55 \text{ mV}$, the neuron is said to be depolarised and an action potential is generated (by the opening of voltage-gated ion channels) [40]. Subsequently for any new action potential to occur there is a minimum delay caused by the time it takes for the potential to hyperpolarise, i.e. return to the resting potential.

When the action potential reaches the end of the axon, i.e. the synapse, neurotransmitters (stored locally at the synaptic vesicle) are released into the synapse. These neurotransmitters are then detected by receptors on the dendrites of a different neuron. If enough neurotransmitter is detected by these receptors, a subsequent action potential is triggered in said new neuron [40]. Although the process described above is a 1-1 process (1 neuron to another), in reality the network of neurons can be much more complicated, with multiple synapses transmitting to a single dendrite and vice-versa.

Neuroplasticity refers to the changes in the brain’s neural pathways and synapses in reaction to its external environment. It is this mechanism that allows the brain to comprehend and adapt to the changing world around it [41]. In particular, synaptic plasticity is the strengthening or weakening of synapses based on their activity, which can result from a change in the number of receptors at the synapse. The principle of Spike Timing Dependant Plasticity (STDP) states that the if the input action potential to a neuron is immediately followed by an output action potential of the same neuron then, this input is stronger. The opposite also holds true, if the input is preceded by an output spike then is said to be weakened. These synaptic changes can be short or long termed. The long term strengthening and weakening of these synapses is called Long Term Potentiation (LTP) and Long Term Depression (LDP) respectively [42].
2.3.3 Types of transcranial Electrical Stimulation

Transcranial electrical stimulation can be applied in different forms as shown in Fig. 2.7, which are discussed below. This study focuses particularly on tACS due to its potential to modulate ongoing neural activity in a frequency, and phase specific manner, giving it multiple parameters that can be adjusted in real-time and thus making it ideal to perform closed-loop operation.

transcranial Direct Current Stimulation (tDCS)

tDCS applies a constant direct current, the electrodes are referred to as the cathode (+ve current) [43] and the Anode (-ve current) [44]. tDCS alters the resting state of the targeted neurons allowing increased/decreased activity depending on the charge of the electrode [45]. Fig. 2.7.1 shows a standard tDCS waveform.

Nitsche and Paulus, [46], are considered the pioneers of modern day resurgence of tES by showing prolonged tDCS, in the region of minutes, produced specific changes in cortical excitability. They showed that anodal and cathodal stimulation increased and decreased cortical excitability respectively, suggesting that anodal and cathodal tDCS facilitates hyperpolarisation and depolarisation of excitatory neurons on cortical areas respectively. tDCS (or any form of tES for that matter) does not directly cause action potentials but rather increases or decreases the membrane potential of neurons thus, providing an initial facilitation of increasing activity and the sustained potential over time can possibly result in a long term potentiation/depression of the synapses [42]. Since then tDCS has been applied to multiple paradigms exploring its effect on behaviour and
furthermore, can be used to confirm the role various brain regions play while performing different tasks, giving a greater understanding of the operation of the human brain. For example, [47] confirm the role of the supramarginal gyrus (located in the parietal lobe) in pitch memory in nonmusicians along with showing the potential of tDCS to enhance pitch memory in said population.

However, for tDCS (and tES in general) there remains some debate about uses, applications, and effect sizes with many studies still ongoing and in academic literature. Though various studies presented use of tDCS to modulate cognitive and behavioural activity both during and after stimulation with clinical applications, see [48]–[51] for reviews; this debate stems primarily from the variability in repeatability of results [52], [53]. There are multiple reasons suggested for the source of this variability. In their review, [48] suggest greater care needs to be taken when designing studies due to the state dependant nature of the efficacy of stimulation. They discuss both task related variability and interindividual differences and the role they may play. Furthermore, [54] refer to low sample sizes in current studies and furthermore, the prevalence of p-hacking (tailoring statistical analysis to obtain significant results) and publication bias (only reporting significant results). They suggest that studies need to increase transparency which can be achieved by pre-registering studies and reporting results regardless of outcome and furthermore making data available publicly. They also support the use of priori power analysis to estimate the required sample sizes to achieve significantly statistical results. Finally, [55] suggest combining the use of Finite Element Modelling (FEM) and MRI imaging to tailor stimulation montages to individual subjects to provide customised stimulation to achieve more consistent results.

The task dependency of tDCS efficacy, as discussed by [48], supports the design of closed-loop stimulation protocols. For example, [56] presented a timing dependant effect of tDCS in explicit motor learning. For a total of 15 subjects performing a learning task, where they were requested to learn sequences of digit pressing, anodal and cathodal tDCS was applied before the task (for 8 subjects) and after the task (7 subjects). Cathodal and anodal stimulation during the task were correlated with slower and faster learning respectively, furthermore both anodal and cathodal stimulation applied before the task slowed learning rates. This suggests a state dependant effect of stimulation, with online stimulation (during task) providing better modulation of behavioural tasks. However with regards to closed-loop operation, only the amplitude of stimulation can be adjusted in real-time when applying tDCS as the other factors (polarity and initial/final ramping of current) are constant after stimulation starts. Thus, limiting the application of tDCS and making it an unsuitable choice for development of the targeted closed-loop system.
**transcranial Random-Noise Stimulation (tRNS)**

tRNS applies a current with parameters of random noise with a broad frequency bandwidth ranging from 0.1-1000Hz [57], see Fig. 2.7.2. It was developed with the goal of interrupting synchronous activity prevalent in cognitive disorders by [58], who showed that this form of stimulation successfully enhances excitability after stimulation over the M1 (motor cortex), in specific stimulation at the higher band of frequency (100-640 Hz compared to 0.1-100 Hz). Furthermore high frequency tRNS (100-640 Hz) has been shown to affect facial perception abilities in both healthy and older adults. A study in 2015 [59] presents the use of tRNS to process social information, showing that tRNS over the occipital cortex increased facial perception ability in healthy subjects in two different experiments (total of 76 subjects). Subsequently, [60] extended these findings by exploring the effect of tRNS in older adults (mean age of 70.1 years for 32 subjects). Subjects performed tests for facial identity perception and facial happiness and anger perception. Their results showed increased performance in anger perception and not the other tasks after stimulation at the inferior frontal cortex, confirming both the role of the inferior frontal cortex in processing anger emotions and, potential use of tRNS to enhance age-related declines in anger perception. They however argue the need to combine their methods with neuroimaging techniques such as EEG/fMRI. This will allow the confirmation of the role of the targeted region rather than the effects being perceived due to a general spread of the current in the frontal lobe and also further explore the task dependent effects of stimulation seen here (no effect of stimulation for facial identity and happiness tasks was observed).

The presented studies show the potential application of tRNS to effect motor and cognitive function in the human brain. The key difference of tRNS from tACS is that the amplitude of the stimulation is rapidly changing due to a constantly alternating frequency. Thus after the depolarisation of the neurons, unlike tDCS, the neurons can hyperpolarise and be ready to fire again [61]. Due to this characteristic, it is thought that tRNS has a better effect in modulating oscillatory neural activity and also a longer lasting effect. For example, [62], showed an improvement in calculation and memory based arithmetic learning with the increase in performance for the stimulation group compared to the sham group still being present 6 months after tRNS stimulation. This suggests that tRNS is capable of producing long term changes in the brain related to neuroplastic reorganisation [48].

Although it has high potential to produce long term changes in cognition and behaviour, the use of tRNS is not ideal for closed-loop stimulation as by its nature the frequency specific information in the stimulation is generated randomly, without any control of the bandwidth of said stimulation.
transcranial Pulse Current Stimulation (tPCS)

A modification of the tDCS protocol is transcranial pulse current stimulation (tPCS), where instead of a direct current a pulse of current is injected like a rectangular wave which stimulates at a particular current intensity and then drops back to 0. This allows for both the sustained hyper/de-polarisation of the neurons in targeted brain regions but also prevents a static build up of charge at the electrodes as well. Furthermore, the width of the pulse can be altered to affect plasticity, see Fig. 2.8. The overall frequency of stimulation usually ranges from 1-100 Hz [63].

In their study, [64] showed that tPCS (short interval) is able to cortically excite the motor cortex more effectively than tDCS. Also, [63] recorded EEG activity before and after stimulation to find an increase in theta and low-alpha correlation between hemispheres, implicating potential applications in memory formation and learning. Furthermore, [63] showed that a random frequency based stimulation ranging from 1-5 Hz increased theta and low-alpha band coherence in comparison to 1 and 100 Hz stimulation. Finally, [65] showed improved response times during an attention switching task after tPCS in the 1-5 Hz range.

However it should be noted, with the exception of [64], who stimulate at the motor cortex, other tPCS studies stimulate using electrodes placed as ear clips. Although in theory, tPCS and tACS can have similar effects, there is little available literature on the method itself with no studies directly comparing the effects of the two different forms. Furthermore, although the pulses of stimulation can be targeted in a phase specific manner to ongoing oscillation, again due to lack of research and by design of the stimulation waveform, tPCS is not an ideal form to directly alter ongoing oscillations in closed-loop, as phase based stimulation is limited to a pulse rather than providing a constant oscillator to effect ongoing frequency-specific activity in the brain.
transcranial Alternating Current Stimulation (tACS)

In tACS, a sinusoidal current signal is applied to the electrodes which allows modulation of rhythmic neural activity to match the stimulation frequency and thus, promoting/disrupting synchronisation within a cluster of neurons [66]. The frequencies are usually in the range of 0.1-40 Hz, though higher frequencies (upto 5 kHz) have also been applied [67].

Unlike tRNS, where the change in frequency is random which leads to rapid changes in stimulation amplitudes, tACS has a consistent and rhythmic stimulation signal which can lead to entrainment of neural activity at the stimulation frequencies [66]. This phenomena can be described as neural resonance, that is the strengthening of neural pathways due to repetitive input depending on the stimulation frequency [68], [69]. Due to STDP (see section 2.2.2) this can result in long term potentiation or long term depression respectively [70]. Thus synapses of pathways with resonant frequencies similar to the stimulation frequency are strengthened by the stimulus resulting in enhanced neural activity. This was shown by a study in 2013, which simulated tACS on a large model of neurons and also an in-vivo study on ferrets [71]. Their results showed that via the mechanism of short term depression (their model did not include/address long term potentiation/depression), the effects of tACS were pronounced as the hyperpolarising phase of the stimulation allowed recovery of the depressed synapses (recently outgoing action potentials), which sets up the next cycle for maximum potentiation.

Also, unlike the other forms of tES, the stimulation waveform for tACS is bipolar with equal intensity in both the +ve and -ve amplitudes, thus the net charge build up at the electrodes will cancel itself out, which can potentially allow safer execution of longer stimulation protocols. Similarly, it does not depend on its polarity to modulate brain activity but on the stimulation frequency [72], allowing direct and easy interference with synaptic plasticity.

For closed-loop stimulation, tACS allows for multiple parameters of stimulation to be adjusted to provide personalised custom therapy. Beyond the modulation of amplitude, the frequency of tACS can be adjusted to exactly match stimulation of ongoing neural osculations. Furthermore, after enabling closed-loop operation, the phase difference between ongoing oscillations and stimulation can be adjusted in real-time to promote or suppress the ongoing neural activity based on requirements of the task. This makes the application of tACS an ideal choice in this study for the closed-loop system. In Section 2.3.4, I discuss various potential applications of tACS as a tool for understanding and modulating cognitive behaviour.
2.3.4 Applications of tACS

Modulation of motor activity

In 2009, a study showed that tACS slowed voluntary movement speeds in healthy subjects [23]. 14 subjects were asked to use a joystick to move the cursor to targeted locations with tACS being stimulated at 20 Hz over the motor cortex. They showed that stimulation slowed down the velocity of the hand at initial movement and when the target was reached, suggesting that stimulation was directly effecting motor execution. They also showed that stimulation in parallel increased beta coherence between scalp recorded EEG activity and Electromyographic activity (EMG) at the hand. Furthermore, studies applying 20 Hz [73], 140 Hz [74] and low kHz range [67] tACS have shown to increase excitability in the motor cortex. An intensity dependant effect of tACS was also observed, where [74] found that stimulation at low amplitudes (0.4 mA) led to inhibition of motor evoked potentials whereas, they also showed that 1 mA stimulation increased excitability. Further investigation regarding the mechanisms behind this effect are yet to be studied.

These findings have been extended to application in Parkinson’s Disease (PD) by recent studies. One study was able to show a decreased beta band variability of fast distal movements in PD patients [75]. 10 patients diagnosed with PD for over 20 months with clinically rated motor symptoms were recruited, also 10 healthy subjects with the same mean age to the PD patients were included as control. Subjects were asked to perform finger tapping movements as fast as possible with their more severely affected hand. Stimulation was applied to the M1 (motor cortex) on the contralateral side (opposite to hand used) at 10 and 20 Hz at 1mA for 15 minutes in between repetitions of the task. Results showed reduced coherence and variations of beta band power post 20 Hz stimulation in PD only and not the control group. Their results suggest pathological alterations in motor-cortical control are more susceptible to frequency modulating effects of tACS.

Another study was able to successfully suppress hand tremors in PD patients by stimulating tACS in a phase dependant manner to ongoing tremors [76]. They started with calculating physical tremor frequency using accelerometers for 14 PD patients and continued to stimulate 2 mA tACS at the these target frequencies & twice these target frequencies. Stimulation was found to vary the amplitudes of the tremors more at the target frequencies that its harmonics. Furthermore, a relationship between the phase difference of stimulation and tremor activity was found and for a subset of 5 subjects stimulation was applied at the optimal phase difference for suppression recorded from the first experiment. They found that on average the tremors were suppressed by 50% using this method. In the past, tremor suppression via electrical stimulation has required expensive and invasive procedures like deep brain stimulation (see section 1.1), and although this technique is not ready for large scale clinical application, it is a very promising step. It
is also one of the first examples of closed-loop operation of tACS albeit with feedback from physical symptoms rather than neurological data; it highlights the potential of using phase dependant information to modulate tACS parameters to achieve strong cognitive effects of stimulation.

*Modulating visual perception with tACS*

In 2014, [77] showed that 10 Hz tACS increased motion discrimination and adaptation on 15 healthy subjects. Subjects’ decision making to determine if the direction of motion of visual stimuli (direction of dots on a screen) was effected after stimulation at 10 Hz. Stimulation was found to increase the sensitivity of subjects to motion discrimination and reduce it to motion adaptation. Also, [78] present the application of gamma band (40 Hz) tACS on anger perception. 47 participants performed one of 3 different tasks requiring them to identify anger perception in different visual stimuli of faces. Results showed that 40 Hz tACS increased anger perception abilities compared to either 10 or 100 Hz tACS. Results confirmed the role of occipital gamma activity in anger perception and show the capabilities of tACS to modulate ongoing neural oscillations, in specific to target cases of reduced occipital gamma paired with decline in facial emotion perception.

Furthermore, [79] demonstrate the role of Individual Alpha Frequencies (IAF) in audio-visual perception in the brain. Stimulation delivered at IAF, IAF+2 Hz and IAF-2 Hz during a double-flash illusion task: the presentation of 2 auditory beeps played in a 100 ms time window paired with a visual stimuli of a single flash in the same time window leads to the illusion of a second flash. Stimulation below and above the IAF was found to shrink and enlarge the temporal window of illusion perception suggesting the role of alpha oscillations in visual perception. In particular, the illusory flash is produced in the temporal window of audio-visual processing linked occipital alpha oscillations and also the ability of tACS to modulate activity in the alpha band to directly affect perception.

*Retinal stimulation using tACS*

tES is also known to induce phosphenes as a side-effect. A phosphenes is a phenomenon characterized by the experience of seeing light without light actually entering the eye [80]. A paper published in 2008 [72], investigated the these phosphenes by stimulating at the occipital and found that the intensity of the observed phosphenes was frequency dependant. Furthermore they found that when stimulating in lighted conditions the most sensitive frequency was 16 Hz (beta band) but when stimulating in dark conditions the most sensitive at the alpha band (10 Hz).

A subsequent study on phosphenes [81], stimulated both at the frontalis and the occipital. Their results showed the threshold to achieve phosphenes was much lower when stimulating at the frontalis, though they did not conclude that the two different montages caused phosphenes from the two different regions but rather that the really high thresh-
Figure 2.9: Results showing the mean threshold for phosphenes and its SEM (standard error of the mean) for the 12 different frequencies (2-24Hz in steps of 2 Hz) for the three different light conditions; DARK (black-square) - 4-6Hz most sensitive, MED Light (red-circle) - most sensitive around 16Hz but less variance over the range and Bright (blue-triangle) - most sensitive at 16Hz. All three have the same threshold around 10Hz and it seems like they trend to intersect again at a higher frequency.

Figure 2.10: transorbital tACS - stimulation around the eye using small ring electrodes

old at the occipital means that diffusal effect of the current by the spinal cortical fluid cannot be discarded. Till now, there remains doubt over the origins of the phosphenes when stimulating at the occipital. They also found that in light conditions (their only condition) that the most sensitive frequency was found to be at 20 Hz. These discrepancies led to the suggestion that maybe the frequency response is quantitatively dependant on the amount of ambient light.

I was part of the group which investigated this theory. Three different light conditions were investigated, it was found that when in completely dark conditions the most sensitive was in the theta band, whereas 16 Hz stimulation was most sensitive with ambient light present. There also some other interesting characteristics (Fig 2.10) in these results which require further study. The motivation behind developing an understanding the characteristics of these phosphenes is the potential development of a non-invasive retinal prosthetic, which will be the first of its kind.

tACS has already shown benefit in application to vision restoration using transorbital

\(^1\)The results for this study have not been submitted for publication yet.
tACS (rtACS), which is the stimulation of tACS around the eye using small ring electrodes (Fig 2.11). rtACS was shown to improve the field of vision for patients with optic nerve damage in a clinical study with 446 patients. To my knowledge this is the first clinical scale application of any form of tACS.

Working memory enhancement using tACS

Studies have shown a trend in the application of tACS to improve working memory performance in healthy subjects [82],[83],[84]. Established working memory models present the roles of theta and alpha activity in working memory capacity and inhibition of inactive regions respectively. The aforementioned studies all apply theta tACS to influence working memory performance in healthy subjects but vary both the tasks (between capacity and executive function tasks) and stimulation montages between targeting the left parietal and left dorsilateral frontal lobe. There remains some discussion of the role these two regions in these tasks as one group got positive results when stimulating at the frontal lobe, whereas the rest of the studies found that stimulating at the parietal was more effective. Thus, [84] suggest that this is because of slight differences in the working memory tasks used by the different groups, explaining that the stimulating at the parietal directly increases working memory storage capacity whereas stimulating at the frontal lobe increases attention thus promoting fluid intelligence.

The application of tACS in working memory is a particular focus of this study, and in Chapter 5, I present methods to monitor working memory during tACS. Thus, a detailed review of both working memory models and the potential role of tACS in enhancing performance is presented in Section 5.2.

Summary

As described in this section, tACS is a strong tool for modulating ongoing neural oscillations in the brain. By design it can be adapted to target specific neural oscillations and has shown to effect functions in the motor, occipital, parietal and frontal cortical regions. Theta tACS has been linked to working memory performances, beta to motor function, and alpha & gamma to processes in the visual cortex.

However a complete understanding of the effects of tACS stimulation is still lacking [85]. It is clear that the efficacy of tACS is directly related to ongoing frequency activity as shown by in-vitro study on mouse neocortical slices; [86] showed that tACS enhanced endogenous stimulation frequencies but not on frequencies that were not active during stimulation. Also, [87] in their review of tACS suggest that studies should aim to target individual endogenous frequencies assessed via EEG/MEG measurements to individually tailor stimulation frequency. This is very indicative of the potential and the need for closed-loop tACS operation to provide customised therapy that produces enhanced cognitive effects.
2.3.5 Safety Guidelines for tES

Introduction

Transcranial electrical stimulation techniques have been used for many years due to their capacity to safely modulate brain activity. To date, there has not been reported any severe adverse reaction [88].

Reported side effects

A paper published in 2007 [89] studied the partially adverse effects of 567 tDCS sessions extending two years. One-hundred and two of these subjects completed a questionnaire asking about the presence and severity of headache, difficulties in concentrating, acute mood changes, visual perceptual change and any discomfort sensation like pain, tingling, itching or burning under the electrodes, during and after tDCS. Of these participants, 75.5% were healthy subjects, whereas the others were migraine patients, post-stroke patients and tinnitus patients. Table 3.1 shows the incidence of adverse effects in healthy subjects. During tDCS, a mild tingling sensation was the most common reported adverse effect in healthy subjects (72.7%), followed by itching sensation (36.4%) and burning sensation (22.7%). In addition, the intensity of the tingling sensation \( (p=0.02) \) and the incidence of tingling sensation \( (p=0.02) \) were significantly higher during tDCS in the group of healthy subjects, in comparison to patients (64% and 12% incidence in the patient group respectively).

Although the most frequent side effect was the tingling sensation, only 17.7% of the volunteers found the stimulation procedure mildly unpleasant. After stimulation, the most common adverse effects in the healthy group were fatigue (24.7%), itching sensation (15.8%) and headache (7.8%). The occurrence of headache was significantly higher in the patient group \( (24\%, \ p=0.03) \) and constituted the most common adverse effect in that group, followed by fatigue (16%), itching sensation (12%) and tingling sensation (12%). The significant difference in the occurrence of headache after stimulation seems to be related to the type of disorder in the patient group.

The results of this study, in which the stimulation was applied over motor and non-motor cortical areas (occipital, temporal, parietal), are in accordance with other published observations. For example, Gandiga et al. [90] found similar side effects such as slight tingling sensation (72.7% of healthy subjects) or transient mild burning (22.7% of healthy subjects) in similar ratios.

The higher incidence of itching, tingling and burning under the electrodes during stimulation that after it suggests that these sensations are associated with the onset of tDCS,
Table 2.3: Reported adverse effects of tDCS in healthy subjects [89]

<table>
<thead>
<tr>
<th></th>
<th>During stimulation (%)</th>
<th>After stimulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tingling</td>
<td>72.7</td>
<td>6.5</td>
</tr>
<tr>
<td>Itching sensation</td>
<td>36.4</td>
<td>15.8</td>
</tr>
<tr>
<td>Burning sensation</td>
<td>22.7</td>
<td>3</td>
</tr>
<tr>
<td>Pain</td>
<td>18.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Headache</td>
<td>3.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Fatigue</td>
<td>35.1</td>
<td>24.7</td>
</tr>
<tr>
<td>Difficulties in concentrating</td>
<td>11.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Nervousness</td>
<td>5.2</td>
<td>0</td>
</tr>
<tr>
<td>Unpleasant sensation</td>
<td>17.7</td>
<td>–</td>
</tr>
<tr>
<td>Nausea</td>
<td>–</td>
<td>3.9</td>
</tr>
<tr>
<td>Acute sleeping disturbance</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2.4: Ratings of discomfort, attention and fatigue in healthy volunteers over 170 tDCS sessions. Discomfort was expressed on a VAS scale of 1-10 (1, representing no discomfort, through 10, representing extreme discomfort/pain). Attention and fatigue were expressed on VAS scales of 1-10 (10 representing most attentive/least fatigued, through 1, representing least attentive/most fatigued). Ratings are expressed as mean±SEM. [90]

<table>
<thead>
<tr>
<th></th>
<th>Pre-Stim</th>
<th>During Stim</th>
<th>Post-Stim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discomfort</td>
<td>–</td>
<td>1.53±0.23</td>
<td>–</td>
</tr>
<tr>
<td>Attention</td>
<td>8.67±0.26</td>
<td>–</td>
<td>8.92±0.31</td>
</tr>
<tr>
<td>Fatigue</td>
<td>8.71±0.26</td>
<td>–</td>
<td>8.86±0.31</td>
</tr>
</tbody>
</table>

and might be related to the current density hot-spots at the edges of the skin-electrode interface.

In the study performed by [90], subjects also rated their perceived discomfort through visual analogue scales\(^2\) (from 1, no discomfort, to 10, extreme discomfort/pain). Additionally, both before and after stimulation, they rated their attention and fatigue on VAS (from 10, most attentive/least fatigued, to 1, least attentive/most fatigued). The results of this study in healthy volunteers are shown in table [90]. The discomfort was found to be minimal (1-2 out of 10), and there was no obvious change on subjective ratings of attention or fatigue.

It is generally noted and accepted that the discomforts experienced during tACS are same as the ones mentioned for tDCS [91] [72] [92] [82]. The first study on the perceived effects of tACS was published in October 2014 [93] though it should be noted that the objective of this study was to improve the development of blinding conditions for sham protocols thus the study was biased (subjects were told what to expect) and any observed side effects perceived were already in the previously accepted limits. They recorded the dizziness, pressure, skin sensations and phosphenes observed by 18 subjects, while varying the frequencies from 2-64 Hz, amplitude from 250 to 1.5 mA and montages at the central, parietal and frontal parts of the brain. The phosphenes were most prominent at

\(^2\) A visual analogue scale (VAS) is a psychometric response scale which can be used in questionnaires. When responding, responses specify their level of agreement to a statement by indicating a position along a continuous line between two end-points, as opposed to discrete scales such as the Likert scale.
16 Hz, when stimulating at the frontal lobe. Dizziness was detected to be the strongest when stimulating at 4 Hz and trending to be stronger towards the parietal. The pressure was independent of frequency and found to be only slightly stronger at the parietal lobe. The skin sensation was independent of frequency and observed to be stronger at the frontal lobe and central sulcus. All affects got stronger with current. Though, these effects were well within accepted/tolerable ranges, as the authors write that sometimes they asked the subjects to really concentrate and look for a particular sensation and yet some did not perceive these effects.

More recently, [88] provided a comprehensive review of tDCS protocols over the last decade. They define an ‘Adverse Effect’ in-line with the guidelines of the FDA and other regulatory bodies, to be the cause of irreversible damage or a persistent impairment/disability that requires intervention. After a review of over 33,000 sessions and a 1000+ subjects, no adverse effects were found to be reported. Although there is less literature available for tACS, [94] in their 2017 review and update of safety and ethical guidelines, indicate that no adverse effects of tACS have been found and the most common discomfort that is reported is headaches for a short duration (minutes) after stimulation. Similarly in their review, [95] also report that no adverse effects of tACS have been observed in its first decade of application.

**Equipment Specifications**

*Stimulator*

The stimulation device should guarantee a constant current density, since current density is the relevant parameter for inducing neural damage, not voltage [96]. If the skull impedance (impedance between the two stimulating electrodes) is unstable, a constant voltage device could result in unwanted changes of current density. In order to prevent any possible neural damage as a consequence of a malfunction, the stimulation device should not be able to produce any current above the safety limit.

The stimulator will be capable of stimulating at a frequency up to 5kHz. This study will aim to explore the effect of stimulation within this range as well. A wide variety of frequencies have been previously investigated, ranging from 5Hz-5 kHz (for e.g. [82] - stimulated up to 2.25mA tACS at 5Hz, [97] - stimulated 1mA tRNS (random noise signal instead of alternating current) at 100-600Hz, [67] - stimulated 1 mA tACS at 1, 2 and 5 kHz).

*Electrodes*

Although electrodes and brain tissue do not come into direct contact in tDCS and tACS, the stimulation electrodes might constitute a hazard for the skin.

Metallic electrode dissolution products, caused by the electrode-skin interface, can produce skin damage and are thus not used. Special electrodes should be used in order to
minimise chemical processes [98]. A conductive substance (e.g. saline/gel) should be used to decrease the impedance of the electrode-skin interface and also minimise chemical processes. Ideally, tDCS should be performed with non-metallic, conductive rubber electrodes, covered completely by saline-soaked sponges [98]. This is commonly practised, although in some cases one may use conductive gel in place of saline, which will have similar effects.

According to [96], heat development under the electrodes can also cause damage. However, this is not likely to happen with low current values and has not been reported to happen in tDCS/tACS studies. Nevertheless, subjects should be told to report immediately any heating of the electrodes.

Another issue related to the electrodes is the presence of tingling and itching sensations during tDCS/tACS studies. Using a mathematical model and computer simulations [99] investigated how the current density in the scalp scales with electrode area. One interesting observation in this study, as pointed out by [100], is the presence of regions of high current density at the edge of the electrodes where the electrode meets the scalp. These hot-spots are expected at the edge of metal electrodes, but in the simulations conducted by Miranda et al. [99] the electrodes were modelled as square sponges, with upper surfaces set to uniform electric potentials. Although these hot-spots are not too important if interested in the current density inside the brain, they might be related to the high number of subjects reporting tingling (70.6%) or itching (30.4%) sensations during tDCS studies, as reported by [89], due to the elevated current density. In any case, this does not constitute a hazard and can be easily solved by increasing the ramp-up time (the time before stimulation reaches its peak current), allowing the skin to get used to the stimulus, for the comfort of the subjects.

Safety screening questionnaire

In order to screen for factors in potential subjects that may predispose for a risk of adverse events during TMS experiments, Keela et al. [101] proposed the Transcranial Magnetic Stimulation Adult Safety Screen (TASS). This questionnaire is based on accepted safety considerations for TMS and consists of fourteen yes or no questions. It can be self-administered or completed with the assistance of the investigator. The purpose of the TASS is not to replace experimenter’s judgement, but to complement history-taking. The decision to proceed or not with an experiment depends on the investigator’s judgement. Therefore, a positive screen does not constitute exclusion from the experiment, but further investigation by the clinician conducting the experiment.

Although the TASS was originally designed for TMS screening, it has been applied to tDCS and tACS due to the similarity of these techniques (for an example see [81]). A modified version of the TASS questionnaire will be used to screen potential subjects.
This questionnaire successfully identifies potential safety problems related to tDCS/tACS. (Questionnaire included in Appendix A)

Ethics Application

This study has been approved by the University Research Ethics Committee at the University of Manchester (Ref: ethics/14263). The ethics approval letter is attached in Appendix B.

2.3.6 Stimulation Guidelines

For the current dose of stimulation, [102] introduced the consideration of the following:

1. Current density (A/cm$^2$)
2. Total charge in stimulated area (C)

Current Density

When discussing the safety of tDCS/tACS protocols, the main focus is usually given to current density, rather than to stimulation strength. Current density depends on the current intensity provided by the stimulator device and the size of the electrodes. It is calculated by:

$$\text{Current density (A/cm}^2\text{) = } \frac{\text{stimulation strength (A)}}{\text{electrode size (cm}^2\text{)}}$$  \hspace{1cm} (2.1)

An excessive current density might harm the subject in different ways, for example it can either damage neural tissue or induce long-term effects in the excitability of the neurones, or it can also damage the skin under the electrodes. [90]

It has been shown that current densities below 25mA/cm$^2$ do not cause tissue damage, even when applying high-frequency stimulation over several hours[102]. However such current densities might cause harmful effects to the skin around the stimulation electrode and can be painful to experience. [98] recommend that a value of 0.02857mA/cm$^2$ should not be exceeded for transcranial Direct Current Stimulation (tDCS). For transcranial Alternating Current Stimulation (tACS) higher values have been often used in research. For example [72] used 0.08333mA/cm$^2$, without uncommon side effects.

In a recent review, [88] provide an update on the safety guidelines for tDCS. Based on reviews of animal tissue studies they suggest that brain injury due to direct current stimulation occurs at 6.3–13 A/m$^2$, i.e. 0.63–1.3 mA/cm$^2$. These values are over an order of magnitude greater than established practice and what will be implemented in my experiments.
Total Charge

The total charge in the stimulated area, which reflects the product of current density and stimulation duration for a whole stimulation session, is also a limiting factor and is given by:

\[
\text{Total charge (C/cm}^2\text{)} = \frac{\text{stimulation strength (A)}}{\text{electrode size (cm}^2\text{)}} \times \text{total stimulation duration}
\] (2.2)

The total stimulation duration is given by the product of pulse duration and number of pulses. Tissue damage has been found histologically at a minimum total charge of 216 C/cm\(^2\) [103]. [98] recommend that maximum total charges of 0.022 C/cm\(^2\) should not be exceeded for tDCS.

In the case of tACS, the net build up of charge is zero since the stimulation is bipolar but a high instantaneous build up of charge can also cause tissue damage and also induce seizures [104]. The charge values to induce such effects are a 1000 fold greater than those that used in conventional tACS protocols and any that were used for experiments for this thesis.

I will be stimulating the tACS protocol at a maximum intensity of 1mA. The size of the electrodes will be 3x3cm, 5x5cm & 5x7cm. I will limit the maximum current density to be less than 0.1mA/cm\(^2\) which means when using the 3x3cm electrode the maximum current amplitude that will be applied is 900\(\mu\)A, i.e. 1.8 mA peak-to-peak current. These values are within the accepted range and are commonly practised.

2.3.7 Summary

To date there have been no reported severe adverse effects of tES [88]. A thorough review of the literature concerning the safety and stimulation guidelines was conducted before deciding stimulation parameters for this study, and subsequently the protocols for stimulation presented in Chapters 4 and 5 were approved by the University Research Ethics Committee at the University of Manchester (Ref: ethics/14263), see Appendix B.

2.4 Summary

Before the design of any closed-loop stimulation operation using tES, it was necessary to gain an understanding of both the neuroimaging methods in question and the fundamentals behind the application of tES. A detailed review of imaging methods used in conjunction with tES allowed me to conclude that EEG is the best modality to use for real-time, closed-loop operation. This is due to the complimentary nature of these two methods in modulating and recording electrical activity in the brain caused directly by the formation of action potentials in groups of targeted (on cortical sites of electrodes)
neurons. Additionally both these methods are relatively easy to set-up and are compatible with out-of-lab use.

There are many different forms of tES available and were discussed. This study chooses to focus on tACS because of its substantial potential in the applications in memory, motor function and perception by modulating ongoing endogenous activity in the brain. This allows stimulation to directly target state dependant activity which is shown to have greater effects of stimulation. Furthermore, there are multiple parameters such as stimulation amplitude, frequency and phase that can be adjusted in real-time during tACS to provide neuro-feedback.

Next in the development of this closed-loop system is dealing with the artifact of tACS recorded in EEG during stimulation. Techniques for removing this artifact are presented in Chapter 3.

References


Chapter 3

Designing techniques for tACS-EEG Artifact Removal

3.1 Introduction

As seen in Chapter 2 (see Section 2.2), both tACS and EEG are non-invasive, portable and relatively low cost. EEG measures electrical activity which reflects changes in the electrical state of neurons and represents the current flow, which is directly modulated when applying tES [1]. Furthermore, due to the high temporal resolution of EEG recordings, these two methods compliment each other very well, and are thereby ideal for creating state-of-the-art closed-loop BCIs, that will allow for real-time, personalised tACS therapy using EEG. However tES introduces a large artifact into simultaneous EEG measurements which prohibits the raw recorded data being used for closing the loop and most existing research compares EEG data before and after stimulation.

This chapter presents the first step in development of this novel BCI; the development and testing of techniques to remove the interference that tACS presents in EEG recordings. In section 3.2, I present methods and techniques for applying EEG and tACS in conjunction, in particular how to ensure that the tACS artifacts do not saturate EEG amplifiers and thus the underlying EEG activity is captured. This is followed by a characterisation of the tACS artifacts, which drive the development of algorithms to suppress this interference.

A detailed review, presented in Section 3.3, covers existing methods for filtering the tACS artifact that manifests in EEG/MEG recordings and their limitations are discussed. With these limitations in mind, I designed 2 new methods for tACS-EEG artifact removal that are described in Section 3.4. These methods have channel independent functionality and compatibility for real-time implementation.
Figure 3.1: EEG data can be recorded before (pre), during, and after (post) tES stimulation. However, tES artifacts in the during EEG data limit current studies to pre vs post analyses. New artifact removal procedures are required to enable pre vs during, and during vs post analyses.

3.2 tACS-EEG artifact characterisation

3.2.1 Introduction

As discussed in Section 2.2, studies investigating neurological effects tES use imaging techniques such as EEG, fMRI and MEG [2], [3], [4]. Due to the presence of significant artifacts, majority of these studies are restricted to a model of analysis which compares the post stimulus data with data recorded before stimulation, unable to utilise any data recorded during stimulation (See Fig.3.1).

Before exploring methods for tACS-EEG artifact removal, it is necessary to understand how EEG and tES interact at the set up level, ensuring that valid data can be collected to enable algorithm based artifact removal. The tACS-EEG montages need to be designed such that they capture the whole artifact and no data is lost due to saturation of the EEG amplifiers. If the EEG amplifier saturates due to the tES interference then no usable data will be collected, and no amount of subsequent processing can recover the wanted brain-related information. This is covered in Section 3.2.2.

Based upon this it is then possible to characterise the EEG artifacts that arise, this is done in Section 3.2.3. Understanding the characteristics of the artifact are critical when designing methods to remove said artifacts. This will allow to set the guidelines and requirements for the evaluation of existing methods and design of new removal algorithms which are presented in Section 3.3.

3.2.2 tACS-EEG compatibility

As mentioned in Chapter 2, each EEG channel consists of an amplifier which has 2 inputs (positive and negative) and one output which is the difference in voltage potential of the 2 input sources [5]. There are 2 common methods for electrode setups for EEG, referential and bipolar. In a referential EEG setup, there are a set number of recording channels (say \( N \)) and a common *reference* channel which acts as the common negative source for all recording channels [6]. There is also a ground channel, the difference in
potential between this and all other channels determines the respective positive and negative inputs to the amplifiers. Thus in a referential setup there are a total of \( N + 2 \) EEG electrodes. Fig. 3.2 shows an example of a 4 channel EEG setup with a 5th electrode used as the common reference (negative) input to each channel.

Whereas in bipolar setups [7], each amplifier has both unique positive and negative terminals, i.e. unlike referential setups, each channel has a different negative input electrode and thus for an \( N \) channel setup there are \( 2N + 1 \) electrodes (2 per channel and one ground electrode). Historically bipolar EEG setups were much more common than they are today. This is largely because in the past EEG amplifiers were not able to sufficiently suppress the 50 Hz mains noise (60 Hz in America) using a referential setup, however this is no longer the case with modern instrumentation electronics. Instead a referential montage is preferred as these recordings can be re-montaged at a later point into any desirable configuration of channels, which cannot be done with a bipolar setup.

The mains noise is suppressed by taking advantage of the fact that each EEG electrode picks up the same mains line interference. Thus the noise is common for all electrode but true EEG is different for each electrode. The output of an EEG amplifier is defined as following:

\[
V_{out} = A_{dm}(V_+ - V_-) + A_{cm}(V_+ + V_-) \quad (3.1)
\]

where;

\[
V_{out} = \text{Amplifier output}
\]
There are two components to the output, the difference and sum between \( V_+ \) and \( V_- \). The desired EEG output is the \textit{differential} component of the equation and thus \( A_{dm} \) is typically set to be very high. The sum component represents \textit{common} signals between the two electrodes, typically mains noise, and thus \( A_{cm} \) is set to be very small (ideally 0 but this is not electronically possible). The ratio \( A_{dm}/A_{cm} \) is called the Common Mode Rejection Ratio (CMRR) of the amplifier [8]. These constant values vary based on the frequency of the input and for most EEG amplifiers, they are optimised to have a maximum CMRR at 50/60 Hz in order to suppress the mains noise.

A common issue when recording EEG and tACS together is the saturation of EEG amplifiers as the artifact due stimulation can be at times larger than input range of the amplifier. EEG activity is usually in the range of ±100 µV whereas with tACS we are applying a current in the range of approximately 1 mA across electrodes with impedances of approximately 5 kΩ. Thus a potential of around 5 V is in principle present, which can potentially saturate the amplifier and thus the underlying EEG data will be lost making any subsequent artifact removal futile. However, the CMRR of the amplifier plays a crucial role when recording EEG during tACS. The artifact is usually not seen in the range of volts, rather around a few hundred millivolts. This is due to the common mode rejection of the amplifier as the tACS artifact is a result of the sinusoid input conducting globally across the scalp, skull and Cerebrospinal Fluid (CSF), i.e. the non-cortical conduction of the injected current. Since this artifact propagates as a common noise across all electrodes, EEG amplifiers intrinsically suppress this noise to a certain extent. Since these amplifiers are designed to have a maximum CMRR at 50 Hz, this does not completely remove the artifact but reduces it in amplitude. Moreover the electrode impedances are not all equal and some of the common mode interference due to the tACS is converted to a differential mode interference and not suppressed. Still, this may not be sufficient for some amplifiers and they may still get saturated.

For this reason and also to further utilise this natural suppression of the tACS artifact, it was decided to adjust the EEG electrode montages used in this study to maximise the \textit{common} tACS noise. As shown in Fig. 3.3, this was achieved by placing the reference electrode on the vertex instead of the mastoid (a traditional position). The reference being in the center of the head rather than to one side results in a similar amplitude of tACS noise on both the reference and recording electrodes and thereby more of the artifact is included during the \textit{common mode rejection} and subsequently less noise is present in the
output. If saturation still persists, the next step is to use a bipolar montage with both the input and output electrodes for each channel close to each other such that the amplitude of the artifact will be similar for both inputs. This maximises the common mode component of the interference: as both electrodes are close together and any delays or phase changes in the tACS signal as it propagates across the head are minimized. For the purpose of my experiments, I found that by placing the reference on the Cz was sufficient to suppress the tACS noise to a level where no saturation was observed.

For the rest of this thesis, tACS artifacts refers to the morphologies in the raw EEG trace after this set up (see Fig. 3.3.B) to ensure no saturation of the amplifier occurs.

![Figure 3.3: Example 8 channel EEG-tACS montages on a 10-20 system. tACS electrodes - Red/Blue, EEG positive input - Yellow, EEG negative input/reference - Black. A: traditional referential EEG setup with reference at the mastoid. B: EEG montage used in this study, reference at vertex (Cz). C: Bipolar EEG montage with individual reference electrodes placed contra-laterally to each input electrode.](image)

### 3.2.3 Artifact Properties

The current injection due to tACS causes a voltage potential, based on the stimulation electrode impedance, on the head which manifests as an artifact in the EEG trace which is much greater in amplitude than actual EEG data as is visible in Fig. 3.4. The artifact characteristics for tDCS have been investigated previously by using Independent Component Analysis (ICA) to isolate and remove them [9], [10]. Unlike the tDCS case, where the artifacts take the form of systematic low frequency noise as reported by [9] and shown in Fig. 3.5, tACS manifests as a sinusoidal signal at the stimulation frequency.

As mentioned previously, the artifact is a result of conduction of the injected current on the non-cortical components of the head and presents globally on all channels. Though some of this artifact is suppressed by the common mode rejection of the EEG amplifiers, it still much bigger than true EEG activity (by a factor of 1000). Using recordings on a single subject, after 12 trials, at stimulation amplitudes of 250µA and 1mA, the signal-to-noise ratio, i.e. the ratio between the root-mean-squares of true EEG data and tACS artifact, was found to be -23dB and -33dB respectively. These are significant artifacts present at frequencies which are active during stimulation and thus simply applying a
Figure 3.4: EEG+tACS recording from a human. Until time 810 s good quality EEG is collected. After 810 s tACS starts and a large interference signal is seen. This artifact needs to be removed in order to allow the EEG from before, during, and after stimulation to be studied.

Figure 3.5: The isolated tDCS independent components. Row 1 & 2: Artifacts due to drifting of tDCS current in EEG electrodes adjacent to tACS electrodes. Row 3: Artifact due to small shift in voltage of stimulator maintaining constant current. Figure taken from [9].

A notch filter at stimulation frequencies will result in loss of crucial information of the ongoing brain activity which can be at the stimulation frequency itself. Furthermore, application of notch filters to remove harmonic distortion at such high magnitudes is not ideal as by design, the bandwidth (frequency range) of the notch filter will in effect be wider than the single frequency of stimulation when applied to remove noise of such high magnitudes.

Although in theory the stimulation is constant and thus any true EEG will be embedded in the envelope of this sinusoid, in practice this is not the case and simply subtracting the sinusoid from the data is not effective for two main reasons. Firstly, the true amplitude of noise at each individual electrode is unknown as it will vary for different electrodes.
since some energy will be lost as the injected current passes through the conductive layers of the skull and the brain. Computational modelling has been implemented to model the spread of tDCS current in the brain and neural networks ([11],[12],[13]), but none of these have extended their findings to include modelling of the tACS current and thus this data is unavailable. Secondly, the impedance of the electrodes are never truly constant and thus multiple factors (such as electrodes drying, blood circulation under electrodes, muscle movements and similar) can result in changes in impedance. This will directly result in a change of stimulator’s output since it is trying to maintain a constant current and will adjust the output voltage based on impedance and thereby modulating the artifact itself.

![Graph showing EEG signal with sinusoidal artifact](image)

Figure 3.6: The true EEG in theory should be present in the envelope of a perfectly sinusoidal tACS artifact.

This is illustrated in Fig. 3.6 which quantifies the changes in amplitude that occur on top of the bulk sinusoidal signal. This is a 100 µVpp trace, which would still dominate over true EEG even if the sinusoidal interferer was fully removed. Similar characterisation of the tACS artifact was presented by [14], where they discuss the modulation of the tACS artifact in EEG and MEG recordings due to ongoing processes such as respiration and heartbeats of subjects. Thus the subsequent non-linear artifacts that this results in, are at frequencies around the target stimulation frequency based on heart rate and respiration rates. However, [15] further discuss these non-linear artifacts, suggesting that though when looked at in EEG/MEG data, these artifacts are indeed present, they originate at the stimulator rather than at the electrode. Meaning, since the stimulator is adjusting its output to maintain a constant current, the changes due to rhythmic processes are captured in the adjusted output of the stimulator.
3.2.4 Summary

Here I have presented the origin of the tACS-EEG artifact. Though the output of the stimulator is much higher than the range of EEG units (approximately 5 V), the resulting artifacts in EEG data are recorded in the range of millivolts. This natural reduction of the artifact occurs due to the common mode rejection in all EEG amplifiers, designed to remove mains noise. Furthermore, EEG montages can be optimised to ensure maximal rejection of the common tACS artifacts on EEG electrodes such that the amplifiers are not saturated and all the physiological data can thus be recorded, albeit it will still have significant artifacts present.

The tACS artifact presents itself as a sinusoid at the stimulation frequency which can be (and usually is by design) at a functional frequency that targets ongoing processes in the brain and therefore simply filtering at this target frequency can result in loss of physiological data. Further characterisation of the tACS artifact also shows that beyond the primary sinusoidal artifact at the stimulation frequency, there is the presence of non-linear artifacts that arise from ongoing processes in the body that can modulate the output of the stimulator and are embedded in the envelope of the sinusoidal signal.

Thus methods for tACS artifact removal need to not filter too aggressively such that the true EEG activity at stimulation frequency is also removed. They should adapt artifact amplitudes individually for each electrode to account for loss of energy during propagation of the artifact to different electrodes via the skull and the brain. Finally, these methods need to account for non-linear changes in artifact due to change in impedance of the stimulating electrodes.

3.3 Existing algorithms for tACS artifact removal

3.3.1 Introduction

Having established the properties of the artifacts to remove and their typical morphologies it is now possible to explore previous methods for performing tACS artifact removal from EEG data. To my knowledge this is a very emerging area, and although some methods have been considered, none satisfy all of the requirements listed above.

The Starstim device (NeuroElectrics Ltd.) also have a built in tACS artifact removal algorithm, but since it is a commercial device, documentation of its artifact removal process was not available to discuss. Also, [16] investigated the effect of tACS during sleep using artifact subtraction (recorded at mastoid) and notch filters to remove tACS artifacts, but no performance analysis of this method was presented. Furthermore, due to a lack of EEG activity at most frequencies during REM sleep, the use of notch filters can
be acceptable but may result in loss of crucial data when considering applications with stimulation at active brain oscillation frequencies as discussed above. Also as mentioned above, Independent Component Analysis (ICA) was used to isolate the tDCS artifact [9], but has not been applied to the tACS artifact removal problem. My preliminary investigations suggest that PCA based approaches outperformed ICA ones. Thus I did not further explore ICA, as it has similar limitations to that of PCA but is less effective.

In this section I discuss the use of PCA, Beamforming and Linear Regression for tACS artifact removal from EEG data. Note that I also though Beamforming is a method employed for MEG, it is in theory also possible to apply on EEG data but its restrictions in doing so will also be discussed.

### 3.3.2 Principle Component Analysis

Principle Component Analysis (PCA) is a commonly used technique with EEG data for applications like noise reduction [17], multi-channel artefact removal [18] and also identification of dominant signals used in operation of BCIs [19]. It has already been used to remove components of interference due to heart beats in EEG during fMRI [3].

In 2014, [2] presented it for tACS artifact removal. They split the recorded EEG data during stimulation into 400 segments and then averaged to construct an artifact template for that was subsequently subtracted from the recorded data. Next, PCA was applied to remove the components of tACS artifacts.

PCA constructs a set of weights, Principle Components (PC), based on the covariance of the EEG channels such that each component is uncorrelated with the others and that each component captures maximum variance of the dataset, excluding the variance captured by the previous component [5]. The first PC will have the maximum variance, that is, the most dominant component of the data, and each subsequent PC will have the next most dominant component of the data. As tACS artifact signals dominate over true EEG, the artifact can be removed by decomposing the multi-channel EEG data set, \(X(i, t)\), setting a number of the PCs to zero, and then reconstructing the EEG.

The PCs are calculated by representing the dataset of \(I\) channels with each channel containing \(T\) time samples as matrix \(X_{I \times T}\). The covariance matrix of this is \(R_{xx}\), with eigenvalues \(\lambda_1, \lambda_2, \dotsc, \lambda_I\) (\(\lambda_1 \geq \lambda_2 \geq \ldots \geq \lambda_I\)) and corresponding eigenvectors \(c_1, c_2, \ldots, c_I\). Also, \(C = [c_1, c_2, \ldots, c_I]\) such that:

\[
C' R_{xx} C = X_{I \times T}.
\]  

Each PC is given by \(\lambda_d c_d\) and the reconstructed signal \(S_{I' \times T}\) is obtained as

\[
S_{I' \times T} = \lambda_i c_i \times R_{d \times I} \times C'
\]  

67
where \( d = [2, 3, \ldots] \) (assuming only the first PC is removed) and \( I^* \) is the number of PCs that are retained.

In the described PCA study [2], there were 59 EEG channels and the first 3 PCs were excluded in the reconstruction. When moving towards mobile, low-channel set-ups; as is the aim for this study, this presents a problem. A typical mobile EEG device does not implement such high channel numbers\(^1\) and there is a high chance that even the first PC will contain relevant EEG data when using low channel counts. Furthermore, their verification relied on artificial mixing of sham data with a simulated artifact, but no justification or verification of this method is presented to suggest that this mixing is representative of actual artifact characteristics observed in tACS-EEG recordings. The authors argue that the effect of increased alpha activity was seen primarily at the occipital regions but the statistical evaluation of spatially separated regions was not presented to compliment this assumption, thus these observations may be the result of placing stimulation electrodes at the occipital and the vertex and thus higher residual artifacts at these regions. No follow up work has ever been presented to disambiguate this, or to use the methods in other experiments. In summary, though the results look promising, a step may have been skipped to evaluate and quantify the performance of the artifact removal method and the assumption of effects during stimulation may have been rushed. This is supported by the debate between [14] and [15] as discussed in Section 3.2.3.

3.3.3 Beamforming Filters for MEG

Beamforming filters are commonly used in sonar and radar for signal detection. They apply a spatial filter that suppress interference from unwanted signals. The filter determines a set of coefficients (equal to number of recording channels) based on spectral powers of a set of active and control temporal windows used to minimise the source power [20].

The first study to present tACS-MEG artifact removal using beamforming filters applied a Linearly Constrained Minimum Variance (LCMV) beamformer filter [4]. They used 102 recording channels with a montage of 306 sensors. The covariance matrix of 2-3s epochs were used to obtain a beamfomer filter with 889 spatial points. The beamformer, similar to the CMRRs for EEG filters, suppress noise that is correlated to all sensors. tACS was delivered at 10 Hz for three conditions; sham (no stimulation), low intensity (50\(\mu\)A) and high intensity (based on perception thresholds such as phosphenes and tingling). They successfully show evoked responses to alpha activity onset and visual/audio stimuli, but there is little evaluation/quantification of performance presented. The observed changes are assumed to be due to stimulation parameters, ignoring the possibility of higher residual artifacts with higher stimulation conditions.

\(^1\)The device used for my experiment was deliberately limited to 8 channels for fast set up with (potentially) vulnerable subjects.
In 2016, [21] presented another method for tACS artifact removal from MEG data, recorded across 275 sensors, using Synthetic Aperture Magnetometry (SAM) beamforming. The authors first used a spherical phantom head model filled with saline and containing two electrodes, thus creating an electric dipole which they set to stimulate at three separate frequencies (1, 11 and 23 Hz). Subsequently they stimulated modulated tACS with a carrier frequency of 220 Hz and was modulated at 1, 11 and 23 Hz. They present reconstruction results with high coherence between the stimulation and sham recordings on the phantom head and insignificant changes in source activity between the two conditions. Subsequently, they repeated amplitude modulated tACS on 4 subjects, with tACS modulated at 11 and 23 Hz during a motor task while recording MEG. Their reconstructed data was successfully able to detect beta activity at the M1. However there remain key issues with the presented results. For the phantom head described here, no characteristics of the simulated activity were presented to compare if they had similar amplitudes to true MEG activity recorded on human subjects. Furthermore, the use of a single frequency oscillation to simulate brain activity is not ideal as true neural activity is not limited to a particular frequency band and thus assuming that the tACS artifact mixes similarly with a single frequency signal and actual MEG data is a big assumption and requires some justification. Also, even though they present entrainment due to stimulation, they acknowledge that stimulating using amplitude modulated tACS does necessarily have the same effect as stimulating using traditional tACS frequencies. The effect of the different stimulation waveforms requires future studies, especially considering studies using high frequency tRNS have presented effects of stimulation as well [22],[23].

A lack of evaluation/comparison of artifact removal techniques is a recurring issues in most tACS artifact removal studies. Beyond this observation, two substantial issues are identified by reviewing the application of beamforming methods. First, the beamformer filters are designed to remove common sources of noise from all sensors. This assumes that the tACS artifact propagates globally and equally across the scalp, which is not the case. This is somewhat negated by using a covariance matrix across a very high spatial resolution (over 100 sensors) to account for changes with spatial propagation, but this leads to the second issue of requiring very high channel counts. As discussed in Section 2.2, MEG is already not a portable method for neuroimaging and furthermore to translate these methods to be applied on EEG data, a high channel count such as this is not available in mobile EEG devices, limiting the application of this method to lab conditions.

3.3.4 Linear Regression - NeuroPrax Device

The NeuroPrax tES-EEG (Neuroconn Ltd.) is a device that offers a built in method for real-time tDCS/tACS artifact removal. The device applies Dynamic Linear Regression to determine a coefficient that is applied to a recording of the inputted tACS signal (from
signal output of the DC stimulator plus) which is subsequently subtracted from the recorded EEG data. Details on how the coefficient is calculated are not presented except the description of the use of adaptive linear coefficients that are varied based on unknown constants. This may be to preserve IP as the algorithm is part of a commercial product. However in the only abstract describing this method [24], the only verifications presented are the presence of blink artifacts (as discussed in Section 2.2.6, these are of very large amplitude and not indicative of true EEG being present) and residual post reconstruction activity at the stimulation frequencies.

In practice, researchers have been slow to adopt this method due to the lack of verification of the performance. It also comes at a high cost (greater than £20k) and is restricted to use only with the Neuroconn DC Stimulator Plus. Furthermore, at the start of each stimulation protocol there is a learning period that is used to determine the initial coefficients. This makes the use of this algorithm for closed-loop stimulation not possible as each iteration of adjustment will result in a subsequent training period as the training requires adjusting of initial parameters which will require the user to stop and restart recordings.

### 3.3.5 Discussion

After a review of the existing methods and studies, a common theme is observed. All studies skip the step of verification and are keen to present observed effects during stimulation. Although all methods have scope with some successful results, they assume consistent and effective performances of their respective methods and continue to present more results. No study compares the performance of their methods with any other technique. Although, [21] take existing methodologies a step further by applying the technique on a phantom head, they do not evaluate the characteristics of their simulated dipole activity and do not compare the mixing of tACS artifacts with their phantom head simulation to that of true tACS-MEG artifacts. They also use single frequency simulations as simulated EEG activity rather than a mix of oscillatory activity that is observed in real conditions. However despite these limitation, this is the first study that presents a step before evaluating in-vivo data. This is a key step in my opinion, before methods are adapted by researchers, it is essential to evaluate the performance of the artifact removal process using standardised tests and comparing performance with other techniques. The first in depth characterization and comparison of tACS artifact removal, using both head phantoms and in-vivo data will be presented in Chapter 4.

With the exception of Linear Regression, the other methods are not channel independent. The other 2 methods require a high number of channels which is not ideal for adoption to out-of-lab applications with mobile hardware. Also PCA is not compatible for real-time implementation as a full data matrix is required to identify the principle component. Though Linear regression is compatible for real-time application, the NeuroPrax
device requires a ‘training period’ dependant on stimulation parameters that can greatly reduce the temporal resolution of any closed-loop feedback.

As discussed in Section 3.2.3, [14] investigated and characterized the tACS artifact showing the presence of non-linear stimulation artifacts which were concluded to be a result of “rhythmic changes of the body’s impedance” due to heart rate and respiration. They suggest that artifact rejection methodologies using PCA, ICA and beamforming filters are not adequate and result in residual artifacts post filtering since these changes in impedance are not accounted for in the methodology. However in reply, [15], suggest that some non-linearity exists and that the side-bands will be observed but beamforming can be adjusted to further suppress common signals to account for these differences. This may result in suppression of some actual neurological data but since it can be set to be common for sham and stimulation conditions, thus when comparing and contrasting between the two conditions, it is possible to accept the lost data as any differences will still be observed. They go on to suggest that the described non-linear interference may be caused by the stimulator, especially when operating at its upper limits (at high impedance/current densities) and suggest that these non-linear effects originate at the site of recording and not stimulation. [14] suggest that the non-linear effects are a result of interference of the ongoing changes in the body’s impedance. As I mentioned before, when describing the characteristics of the artifact, the stimulator attempts to maintain a constant current output. Thus changes in impedances result in a change in the voltage output of the stimulator to maintain the constant current and, the resulting artifacts are abruptly changed whenever any significant changes in impedances occur, which may result in residual artifacts when using methods which assume a constant/common noise. Apart from the NeuroPrax, all methods discussed are non-parametric, despite the fact that is a parametric situation. The artifact source/shape is known in principle and with modern hardware can be recorded and used to improve the signal processing performance.

3.3.6 Summary

Previous studies investigating methods for tACS artifact removal were presented and discussed. A review of these methods revealed the following shortcomings in current practices:

- Applied offline
- Low temporal resolution
- High number of channels required
- No existing standard for testing performance
To achieve closed-loop operation of tACS using EEG activity during stimulation it is necessary for new methods to be able to operate in real-time. Current methods are all designed for offline operation and have very low temporal resolution. A lower resolution in the time domain directly affects the rate at which any closed-loop operation can be applied as analysis of ongoing activity is required to adjust the stimulator output. Furthermore, the focus of this study is to provide real world, mobile, out-of-lab BCIs that utilise this closed-loop functionality which is not possible when the required number of channels is very high. A high channel count increases the time required to setup these units and further, most mobile EEG devices have limited number of channels and ones with enough channel counts are too expensive to distribute for large scale medical applications.

3.4 Two novel algorithms for tACS-EEG artifact removal

3.4.1 Introduction

With an understanding of the limitations of existing methods, I will now present two new algorithms for tACS artifact removal of EEG data that I developed over the course of this study. These methods are designed to operate independent of channel count, are easily adapted for real-time implementation with close-loop operation in mind.

3.4.2 Superposition using Moving Averages

The tACS interference signal manifests in all EEG channels. In each channel the amplitude of the interference is different but the frequency of the stimulation is global throughout the duration of the recording and, any phase difference that exists is between channels but individual samples from each channel have no phase differences. This is similar to the periodic artifacts seen in EEG data recorded during fMRI and approaches using removal of moving averages has been applied in fMRI studies previously [25], [3].

A new approach for tACS artifact removal on sliding moving averages was developed by me [26]. The procedure, termed Superposition of Moving Averages (SMA), is illustrated in Fig. 3.7. This was devised to use the known information of the signal, that it is approximately sinusoidal, but with an amplitude that changes over time as the stimulator output varies in accordance with the varying impedance of the stimulation electrodes.

The EEG data set, $X(i, t)$, where $i$ is the channel number and $t$ is time, is first split into non-overlapping segments such that the length of each segment matches the period of the tACS stimulation frequency. If the period of stimulation cannot be split into segments with an integer number of samples the segment length is set to be as small as possible while also being periodic and an integer number of samples. For example, the seg-
Data represented as $X(i,t)$ where $i = [1, 2 \ldots 8]$, represent the EEG channels.

Each channel split into $N$ segments, lengths based on the stimulation frequency.

Moving average of each segment and $\lceil M \rceil$ neighbouring segments is calculated.

Averages of segments are concatenated to form artifact template $[A(i)]$ of each channel.

Artifact template subtracted from original data to provide cleaned EEG.

$X(i,t) = [y(1), y(2), y(3) \ldots y(N)]$

$[N = \text{number of segments}]$

$Y(n) = \frac{1}{M + 1} \sum_{n-M/2}^{n+M/2} y(n)$

$[n=1,2,3 \ldots \ldots \ldots N]$

$A(i) = [Y(1), Y(2), Y(3) \ldots Y(N)]$

$S(i) = X(i) - A(i)$

Figure 3.7: SMA artifact removal process and algorithm. A time localized artifact template is generated for each channel and subtracted from the recorded data.

ment length for one period of 40 Hz stimulation sampled at 500 Hz is 12.5 so 25 samples, two periods of the stimulation frequency, are used.

Each segment, $y(i, n)$, where $n$ is the epoch index, and its $M$ neighboring segments are then central averaged to create a time and channel dependent local artifact template $A(i, n)$. This artifact template is subtracted from the data and the resulting signal after un-segmenting the signal, $S(i, t)$, represents the underlying EEG data during stimulation. As the artifact template is specific for each EEG channel this approach can map to an arbitrary number of channels without degrading performance. In this work $M$ is selected to be 5% of $N$ (the total number of segmented epochs) as a suitable trade-off between the number of averages taken and the time localization of the artifact template within the one minute stimulation period.
3.4.3 Adaptive Filtering

Adaptive Filtering (AF) is a parametric time varying filtering approach [27]. As opposed to conventional filters which have fixed filter coefficients the AF filter coefficients are varied depending on the accuracy of previous filtering iterations using an optimization algorithm and error cost function derived from the input noisy signal (EEG+tACS) and an estimate of the interfering source (tACS) [27]. This approach has been widely used for noise-canceling headphones where the noise signal (wanted audio+background noise) is separated from an estimate of the interference (background noise) recorded using a microphone [28]. If an estimate of the tACS artifact is known the EEG+tACS artifact system can be thought of as equivalent to this. An overview of our application of this principle is shown in Fig. 3.8.

It is possible to record the output of some tES stimulators, including the one used here, as an analogue voltage signal which can then be used for the adaptive filter as the known noise \((d[i])\). The amplitude of this recorded tACS signal is adjusted by multiplying it with the root mean square of the EEG data at a given channel. This forms one input \((d[i])\) to the AF, with the second input \((x[i])\) being the EEG recording, which is a mixture of the true EEG \((a[i])\) and the tACS signal \((d[i])\).

For formulating the adaptive filter, several different methods are available and from preliminary work I selected the RLS algorithm [27]. This method utilises a weighting factor to minimise the error estimates which can be manipulated by adjusting the value of a constant called the forgetting factor, \(\lambda\) (a positive constant in the range of 0 to 1). By selecting a small value for the forgetting factor the cost function puts more emphasis on recent values of error estimates (forgets the past) whereas a value closer to 1 increases the memory of the algorithm and hence it includes older estimates when determining its coefficients. A system with higher memory intuitively fits the case of this setup where the tACS signal is periodic and thus deterministic (not random) and consequently produces a more stable output when fed into an RLS filter with a high forgetting factor.

Linear adaptive filters such as the one described above operate on the principle of minimisation of the error square and for the RLS adaptive filter this objective function is defined as:

\[
E = \sum_{n=1}^{N} \lambda^{n-j}[e(n)]^2
\]  

(3.4)

Where:

\(E\) = error square;
\(\lambda\) = forgetting factor;
\(e(n)\) = the error output as defined in Fig. 3.8.
Figure 3.8: Adaptive Filtering artifact removal process and algorithm. The stimulator output of the tACS output is used to dynamically set the artifact removal filter coefficients.

As described in Fig. 3.8, for inputs signal \( x(n) \) the output signal \( y(n) \) (where \( n = 1, 2, 3, \ldots, N \) is the number of inputs/outputs) is a product of the previous input signal with a weighting function \( w(j) \) where \( j = 1, 2, 3, \ldots, J \) is the number of filter coefficients and the error signal is defined as:

\[
e(n) = d(n) - y(n)
\]

where \( d(n) = \text{desired signal} \).
Thus, Eq 3.4 can be rewritten as:

\[
E = \sum_{n=1}^{N} \lambda^{n-j} [d(n) - y(n)]^2
= \sum_{n=1}^{N} \lambda^{n-j} [d(n) - \sum_{j=0}^{J-1} w(j)x(n - j)]^2
\]  

(3.5)

Now if we define \( \bar{x}(n) = \sqrt{\lambda^{n-j}}x(n) \) and \( \bar{d}(n) = \sqrt{\lambda^{n-j}}d(n) \), Eq 3.5 now becomes:

\[
E = \sum_{n=1}^{N} [\bar{d}(n) - \sum_{j=0}^{J-1} w(j)\bar{x}(n - j)]^2
= \sum_{n=1}^{N} \bar{d}^2 - 2 \sum_{n=1}^{N} \bar{d}(n) \sum_{j=0}^{J-1} w(j)\bar{x}(n - j) + \sum_{n=1}^{N} \sum_{j=0}^{J-1} w(j)\bar{x}(n - j)\bar{x}(n - j)
\]  

(3.6)

By rearranging the linear summation on the last term of the RHS, we can rewrite it as:

\[
\sum_{n=1}^{N} \sum_{j=0}^{J-1} w(j)\bar{x}(n - j)\bar{x}(n - m) = \sum_{j=0}^{J-1} \sum_{m=0}^{J-1} w(j)w(m) \sum_{n=1}^{N} \bar{x}(n - j)\bar{x}(n - m)
\]

Now, let’s define the following:

\[
\begin{align*}
    r_a(j - m) &= \sum_{n=1}^{N} \bar{x}(n - j)\bar{x}(n - m) \\
    r_c(j) &= \sum_{n=1}^{N} \bar{d}(n)\bar{x}(n - j)
\end{align*}
\]  

(3.7)  

(3.8)

Now, subbing Eq. 3.7 and 3.8 in Eq. 3.6 and rearranging, we get:

\[
E = \sum_{j=0}^{J-1} \sum_{m=0}^{J-1} w(j)w(m)r_a(j - m) - \sum_{j=0}^{J-1} w(j)r_c(k) + \sum_{n=1}^{N} \bar{d}^2
\]  

(3.9)

Now, Eq. 3.9 resembles a quadratic equation of the form:

\[
E = ah^2 - 2bh + c^2
\]  

(3.10)
Where, \( a \) is \( r_a(j) \) and represents the autocorrelation function of \( x(n) \) and \( b \) is \( r_c(j) \) and represents the cross-correlation between the desired output \( d(n) \) and the input signal is \( x(n) \) \[29\]. Finally \( c^2 \) and \( h \) are the desired signal and a given filter coefficient respectively. The filter operates by adjusting values of \( h \) such that the error is minimised and an ideal solution may be achieved by solving the differential of Eq. 3.10. However in reality this is not possible as this assumes that the autocorrelation and the cross-correlation functions can be calculated which are not necessarily available in practical conditions \[29\].

Thus in practice the RLS filter achieves this by continually adjusting filter coefficients, \( w(j) \), to minimise error. After setting an initial value for the coefficients, usually zero, each successive input \( x(n) \) is used to generate the output signal \( y(n) \). As described in Fig. 3.8, the difference between the output and the desired signal \( d(n) \) is defined as the error, \( e(n) \).

Now the coefficients are adjusted based on this error signal as defined in Fig. 3.8. The way this works is that if the error is zero, the filter is not changed. However if there is an error, the coefficients are adjusted by adding to the previous filters a fraction of the error. This fraction is determined by applying a gain, \( k(n) \) to the error. This gain, as defined in Fig. 3.8 is determined by the forgetting factor \( \lambda \). If \( \lambda \) approaches zero, the gain will be determined by only the previous estimate as the intermediary vector \( u(n) \), which contains the recursive properties, will cancel out from the gain \[27\]. Whereas a higher value for the forgetting factor will add more weight to the previous iterations, thereby increasing the memory of the filter.

RLS adaptive filters are also known for having a much faster convergence (stabilisation of output) at a cost of higher computational complexity, which is expected when the algorithm is required to retain previous estimates \[27\]. A limitation of this method is that only a single tACS trace is recorded directly from the stimulator, but in practice this trace will be slightly different at each electrode site due to small phase delays as the signal propagates. In future, with better modelling techniques, these delays can be accounted for but currently no such methods exist. The recorded EEG signal during stimulation for each channel is individually sent to the adaptive filter. Thus similar to SMA, the adaptive filtering is independent of the number of EEG channels and is time varying and thus able to adjust based on the changes in the recorded artifact signal over time.

### 3.4.4 Summary

Both new methods presented in this section have low temporal resolution and are compatible for real-time operation. SMA is only limited in temporal resolution by the width of the moving average, i.e. the number of windows averaged for each epoch which can be adjusted for ideal operation based on the design of particular experiments. The Adapt-
tive filter can also operate in real-time and the number of coefficients will determine the
temporal resolution, where the processing time of the filter is directly proportional to the
number of coefficients used. Each of these methods are independent for each channel
and are thus ideal for operation using mobile EEG devices with low channel counts. The
next step is to test and verify the performance of these methods which are presented in
Chapter 4.

3.5 Summary

Methods such as PCA, Beamforming and Linear Regression have shown success in tACS
artifact removal, there remain key limitations in these methods. The two new methods
proposed in this chapter aim to address these limitations.

Both PCA and Beamforming assume the tACS artifact to be constant noise sources which,
as discussed in Section 3.3.5 and presented by [14], is not the case. The output of the
tACS stimulator is always adjusting to maintain a constant current based on the changing
impedance of the stimulation electrodes. SMA and adaptive filtering, both account for
these. SMA is taking a moving average of the noise template at each channel and thus as
the noise output from the stimulator varies, it will be reflected in the average noise tem-
plate that is calculated. For the adaptive filter, the noise template is the direct output of
the stimulator and is used as input to the filter. Thus the adaptive filter intuitively takes
this into account when reconstructing the EEG data.

A systematic lack in literature was revealed in methodologies applied to gauge perfor-
mane of these artifact removal methods. All existing studies present their respective
methods, the principle behind them and then EEG/MEG data after artifact removal. How-
ever, there remains a missing step in these findings. No measure of performance or com-
parison to previous methods is presented. Subsequently considerable debate remains on
which method is effective and researchers designing experimental protocols are unsure
of which method to apply.

Thus, in Chapter 4, I will present methods for testing of the performance of the 2 novel
methods using a phantom head model designed specifically for this purpose. Further-
more, I will then verify these performances using on-subject data and compare with an
existing method to establish a level of confidence in the presented results which is previ-
ously lacking in literature.

References

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Chapter 4

Testing and verification of tACS-EEG artifact removal

4.1 Introduction

As discussed in Section 3.5, studies presenting methods for tACS-EEG/MEG artifact removal skip a crucial step of verification and comparison of their methods with existing ones. This is due to a lack of available techniques to do so, since the paradigm of confirming the validity of the reconstructed data is complex in its nature. The underlying brain state is during stimulation is unknown and the direct physiological effect of stimulation on this state is also unknown.

In Section 4.2, I present the use of a novel phantom head model used to test the performance of the two new methods that I designed for tACS-EEG artifact removal. After testing with the phantom head model, the next step is to collect in-vivo data to verify and test the developed artifact removal methods. As mentioned in Section 3.3.5, existing works present artifact removal but focus on behavioural results. There is a lack of evaluation and no study to date compares the performance of their methods with pre-existing techniques.

Thus in Section 4.3 I will present the application of artifact removal using three different methods and multi-stage systematic methods to verify and compare the performance of these methods. This is the first study to present on-person in-depth analysis of tACS artifact removal. The goal of this is to set a standard of practice that future studies can reproduce to test and compare new methods.

4.2 Phantom Head Model Testing

4.2.1 Introduction

In this section I present the use of a novel phantom head model designed specifically to test the performance of the tACS artifact removal performance. It was designed to simu-
late pre-recorded EEG activity to allow a baseline activity to be recorded thus the reconstucted data can be directly compared with the simulated data to gauge performance.

In Section 4.2.1, I provide brief detail of the previous methods using phantom heads in tES artifact characterisation. In Section 4.2.2, I present details on the design of the phantom head model, the methods to test and verify the performance of the two novel methods, SMA and adaptive filtering, using the phantom head and the data analysis methods used for this. In Section 4.2.3 the results of the testing using the phantom head model are presented with a summary of said results in Section 4.2.4.

4.2.2 Use of Phantom head models with EEG

To test the artifact removal procedures a gelatin phantom head model was prepared. Similar phantom heads have been used in EEG source localisation and artifact characterisation studies ([1], [2], [3]).

In their study, [4] use a multilayer Agar based phantom head to compare distribution of current during tES with computational simulation methods. However, the methodology presented in this study is a step-up from existing phantom head models used for EEG/MEG artifact characterisation in the field of tES.

Studies report use of melons as phantom head for tACS [5] and TMS [1] artifact characterisation respectively. Also as previously mentioned, [6] report the use of a dipole phantom head for amplitude modified tACS artifact characterisation but no details on the structure and composition of the phantom are presented. Also, unlike [6], the phantom head presented here simulates pre-recorded EEG data from actual subjects. This is crucial when testing tACS artifact removal methods as they provide a baseline activity of actual EEG data to compare and quantify the filtered EEG data.

The model used in this study was designed in collaboration with authors of [7]. The authors had previously used an agar based, laminar structure to characterise the performance of 3-D printed dry EEG electrodes. The same model was further developed to be constructed in the shape of a human head based on obtained scans made with the same gelatin structure used previously. Further details of the design and construction of the phantom head model are presented in Section 4.2.3.

4.2.3 Methods

Head Model Design

To give a realistic head shape for the positioning of electrodes, a head scan was obtained from a grabcad design of human dummies used for automotive testing (https://grab-
The head design was adapted for 3-D printing by creating an inverse shell of the scan using Solidworks. The model then was assembled using an 8-part 3-D print. Subsequently, a 15% gelatin solution (based on previous characterisation of the laminar model by [8]) was poured into the inverse shell and was then refrigerated for 8 hours. The shell was then removed, leaving behind the gelatin phantom head model. Fig. 4.1) shows the different staged of the design and build process of the phantom head model.

Furthermore, two Ag/AgCl electrodes were placed inside the head model to input pre-recorded EEG data. Pre-recorded EEG data was inputted to a bi-phasic current generator (Digitimer, DS4), which stimulated the inputted waveform as a current trace through the head phantom. This EEG shape manifested on the surface of the phantom as a voltage, which could be recorded using a standard EEG amplifier. The simulated EEG activity was subsequently recorded using a single channel EEG setup with the reference (Cz), ground (Cz) and recording (P4) electrodes placed on surface of the phantom head. Finally, tACS electrodes were placed on the head model with the same montage as in–vivo experiments.

To create realistic tACS-EEG artifacts for the simulation, the signal-to-noise-ratio (SNR) of the simulated EEG data and tACS artifacts was matched to those of EEG data recorded during stimulation of the in–vivo experiments. This was calculated by taking SNR between the Pre stage data and the During stage data for all stimulation protocols for all subjects. The average SNR for the 250 µA and 1 mA stimulation amplitudes was found to be −23 dB and −33 dB respectively. Fig. 4.2 shows the artifact manifest at the 30s mark during a recording on the phantom head model (1 mA tACS at 10 Hz) and this artifact can be compared with the real actual artifact seen in Fig. 3.4.
Figure 4.2: tACS artifact recorded on phantom head model during 1 mA tACS at 10 Hz. Clean EEG is seen till the 30s mark when stimulation begins after which the large sinusoidal interference due to tACS dominates the recorded data.

The current intensity of the simulated EEG data was then adjusted such that the SNR matched those of in–vivo recordings. The tACS stimulation settings for testing the on the phantom were kept the same as in–vivo experiments. For testing purposes, one face/non-face ERP task and one alpha inducing task (similar to EEG recordings presented in Section 2.2) recordings for 5 different subject were used as simulated EEG, see Section 4.3.2 for more details of the tasks. For each of these recordings, tACS was stimulated for 60 seconds at 250 µA and 1 mA and at stimulation frequencies of 5, 10 and 40 Hz. Also, for each of the simulated recording, EEG activity was recorded with the absence of tACS stimulation. This provides baseline activity to compare with the artifact removed data.

In Summary, a total of 10 sham recordings (one alpha and one ERP task from each subject) were simulated and 60 stimulation recordings (6 different tACS conditions for each sham simulation).

Data Analysis

The artifact removal on data collected from the head model was applied using SMA and Adaptive filtering to test the performance of these novel methods. The collected data was filtered using 3rd order low pass and high pass Butterworth filters with cut-offs at 50 and 0.5 Hz respectively. Then SMA and AF were applied to stimulation data. The use of the phantom head allows for direct comparison between sham and stimulation data since the sham data presents a baseline for comparison of the artifact removal methods. To quantify this comparison, correlation coefficients were calculated between each stimulation condition and their respective sham condition using the ‘corr2’ function in Matlab (Mathworks, USA).
4.2.4 Results and Discussion

Reconstructed EEG data after 1mA tACS at the three different stimulation frequencies is plotted along with the respective pre-recorded alpha task data in Fig. 4.3. The EEG traces are very similar for both sham and data after artifact removal. Similarly, Fig. 4.4, shows the average reconstructed ERPs for all tACS conditions applied on one sham face/non-face task sham simulation on the phantom head. In each case, the N170 trough for Face the stimulus is clearly detected. However it is noted that the results are noisier when applying the adaptive filter for 1mA stimulation at 10 & 40 Hz.

Figure 4.3: SMA (blue), AF (red) and Sham (black) data recorded on the phantom head when stimulating at 5 Hz (top), 10 Hz (middle) and 40 Hz (bottom) for three different EEG simulations.

Figure 4.4: Average Face (black) and Non-Face (red) ERPs for all stimulation conditions reconstructed using SMA and RLS adaptive filter recorded on the phantom head. All ERPs presented are for tACS applied on the same sham simulation.

The advantage of using a phantom head with simulated EEG is the presence of base-
line activity for comparison with data after artifact removal. High average correlation coefficients (all >85%) between sham and reconstructed EEG data for all stimulation conditions were observed, in comparison the average correlation coefficients between EEG data and data without artifact removal was recorded to be 2.7% for all conditions. Fig. 4.5 represents these for the two different stimulation amplitudes using the two different methods of artifact removal across all frequencies. Although none perform noticeably better than the other, it is observed that the results from the RLS Adaptive Filter have a higher variance. Regardless, these high correlation factors, across all conditions, provide confidence that the SMA and AF methods reconstruct data that captures underlying EEG activity during stimulation.

![Figure 4.5: Correlation coefficients between sham and reconstructed EEG data, collected using the phantom head, after tACS artifact removal using SMA and RLS at the two different stimulation amplitudes (0.25 and 1 mA) across the 3 different frequencies.](image)

**4.2.5 Summary**

To my knowledge, this is the first study that uses a model with simulated EEG activity that provides a baseline to compare reconstructed data in tES. These findings allow future Brain-Computer Interface (BCI) studies to explore the use of tACS to enhance/suppress particular waveforms and responses in order to obtain better BCI accuracy.

The use of a phantom head that simulates actual EEG activity to test these methods is a crucial step. The ability to remove tACS artifacts with a known baseline, allowing di-
rect comparisons and correlation measurements, provides a tool for different researchers to test existing and new methods using their own setup. This can lead to a standard in the field for testing and comparing methods and will successively result in more replicable studies. However it should be noted that the non-linear changes due to changes in impedance presented by [9] are not replicated by the head model. Future iterations of the model can be adapted to artificially simulate these changes to recreate these non-linear artifacts in simulated results by actively changing the impedance of the head model in a controlled environment.

4.3 Verification using on-subject data

4.3.1 Introduction

Here, I present the application of 3 different artifact removal algorithms: Principal Component Analysis (PCA), Superposition of Moving Averages (SMA) and Adaptive Filtering (AF). Data was collected from 5 subjects, while performing an alpha test to induce alpha activity and a facial recognition task to detect N170 ERPs during tACS stimulation at different frequencies and amplitudes while EEG was recorded. This is the first study to apply different methods of artifact algorithms to compare performance of these methods on the same dataset. An 8-channel EEG montage was used for collecting data during tACS stimulation. This setup was intentionally used to compare the performance of the three artifact removal methods on a low channel count to evaluate their use for out-of-lab applications in future.

Furthermore, unlike the phantom head data from Section 4.2, there is no baseline to compare the reconstructed data with. Therefore a multi-stage approach was applied that compared EEG descriptive statistics, observed frequency domain features and detection of the N170 ERPs. This comprehensive strategy provides a degree of confidence on the performance of the artifact removal techniques that was previously missing in previous studies.

The stimulation duration (dosage) was intentionally limited to ensure no significant behavioural changes are observed. The goal of this study was not to report effects of stimulation but to focus on the performance of the different methods as tools for artifact removal during tACS.
4.3.2 Methods

Introduction

For in-vivo tests, demonstrating that the artifact removed EEG is in fact true EEG, and not EEG with residual artifacts present, or an entirely different non-EEG signal, is challenging because by definition there is no true EEG baseline case that the artifact removed EEG can be compared to. I have used a multi-stage and comprehensive testing strategy to not rely on any one set of experiential evidence, but to use several separate analyses to build confidence in the artifact removal process and show that the EEG it produces is indeed true brain related information. This is in contrast to other methodologies for verifying EEG/stimulation artifact removal such as [10] which only visually inspected time and spectral domain data, and [11] which visually inspected spectral data and compared one EEG descriptive statistic. This methodology combines detecting alpha activity, recording changes in Individual Alpha Frequency between eyes open and closed during tACS, detecting ERPs and measuring N170 depths during tACS, and statistically comparing four EEG descriptive statistics to verify the artifact removal process as comprehensively as possible.

Experimental Protocols

To comprehensively compare and determine the performance of the novel artifact removal techniques, two different experimental protocols were considered using EEG+tACS data. These demonstrate that both free-running background EEG (alpha activity in our case) and evoked responses (N170 face sensitive responses) can be successfully measured in the presence of tACS stimulation at different amplitudes and frequencies. Both protocols were 2 minutes long and were repeated 8 (Protocol 1) and 12 (Protocol 2) times. Thus the total experiment run time was 40 minutes for each participant.

All experiments followed the same design, shown in Fig. 4.6. The experimental tasks/stimuli and tACS stimulation only occurred in the 1 minute long During stage, while in the Pre and Post stages, both 30 seconds long, subjects were asked to relax and do nothing so a baseline EEG recording could be established for potential comparisons and to allow a minimum gap between stimulations. The stimulation duration was set to a short duration of 1 minute to avoid inducing long-term changes in the underlying brain state so that descriptive statistics could be applied and compared between the three experiment stages (details of descriptive statistics of EEG presented later in this section).

Protocol 1 – Free-running EEG (alpha task)

In the During stage subjects were asked to keep their eyes open for the first 30 seconds and closed for the next 30 seconds, with directions given verbally. This allowed for spon-
Figure 4.6: The Pre and Post stages are 30 seconds long with no stimuli presented. The During stage is a minute long task/stimuli which is presented during tACS stimulation or during a sham where the same task is performed but no tACS is applied.

taneous alpha activity to be detected at the point when the subjects closed their eyes. It gives a known and sustained natural brain response at can be observed and used as a measure of performance of artifact removal. There were 8 repetitions of this protocol, two sham cases and 6 stimulation cases with different tACS frequencies and amplitudes listed below.

Protocol 2 - Evoked EEG response (visual N170 task)

This protocol was designed to produce P100, N170, and P300 evoked responses which are associated with facial recognition [12] and give known, and very low amplitude (approximately 10-20 µV) EEG components to demonstrate recording and detection despite the presence of a very large tACS interference signal which is in the scale of millivolts. The N170 evoked response is also very commonly used in BCIs [13], [14], demonstrating the potential for using tACS in future BCI set ups.

Subjects were asked to look at images of celebrity faces to trigger recognition and also a non image which was made as a pixelated scramble of the celebrity face images, see Fig. 4.7. In the During stage there were 30 different stimuli presented (15 face and 15 non-face stimuli) in a randomised order. In each run an image would appear on the screen for 1 second and there was a 1 second pause between each stimuli where the screen was blank. The protocol was repeated 12 times for each participant, 6 sham and 6 stimulation cases.

Figure 4.7: Face and non-face images were shown in a randomised order for 1 second at a time followed by a 1 second pause with a blank screen. This figure shows an example of a randomised face, non-face, sequence.

tACS stimulation

tACS was applied using two rubber electrodes placed in saline soaked sponges on FP2 and P3, the electrode postions were selected to mimick previously used montages in ex-
periments looking at effects of parietal stimulation in working memory which implement a contralateral frontal-parietal tACS montage, for example [15]. The stimulation current was delivered using an isolated, battery-operated stimulator (DC–stimulator plus, Neuroconn, Germany).

To demonstrate artifact removal in a range of stimulation cases three different frequencies (5, 10 and 40 Hz) were used for stimulation each at amplitudes of 250 $\mu$A and 1 mA peak-to-peak. These were selected to be representative of high and low current stimulation values used in the wider tACS literature. The stimulation duration was 60 seconds for all 6 different conditions with a 1 second fade in and out of the stimulation amplitude at the start and end of stimulation. The fade in and out of the tACS stimulation is applied to stimulation parameters to prevent itching and burning sensations felt at the start and end of stimulation. The stimulation frequencies of 5, 10 and 40 Hz were used to cover a wide range of EEG bands, and particularly 5 and 10 Hz stimulations were of interest due to their application in working memory performance and because they overlap (or near-overlap) with common cortical frequencies allowing demonstration of the feasibility of acquiring EEG during tACS, even with overlapping frequencies of interest.

The duration of stimulation was deliberately kept short (1 minute) in order to prevent lasting entrainment of neural oscillations such that brain changes due to stimulation are minimized. The aim of this study is to introduce as few brain changes as possible to allow EEG before, during and after stimulation to be compared for the presence of artifacts. To my knowledge no behavioral changes due to tACS have been reported for tACS stimulation durations less than 10 minutes, and no enhancement at any of the tACS stimulation frequencies were observed post stimuli, implying success in not inducing brain changes with the short stimulation. Once the performance of the artifact removal has been established, future studies can be designed to target the behavioral effects of tACS and investigate the electrophysiological effect of tACS during stimulation.

EEG data collection

EEG was recorded during tACS stimulation and for 30 seconds prior to and post stimulation. EEG data was acquired at a sampling frequency of 500 Hz using an 8 channel wireless EEG device (Enobio, Neuroelectrics, Spain). Electrodes were placed at Fp1, F3, C3, C4, P4, PO7 and PO8 with the reference and ground electrodes placed next to each other at Cz. Electrodes were placed directly on the scalp using an adhesive EEG paste (EC2 Electrode Cream, Grass Technologies, USA). This EEG montage is designed to allow recording of EEG data without saturation of the amplifiers due to the tACS artifacts as discussed in Section 2.2.
Participants

To demonstrate the algorithm performance 5 participants were recruited, 3 male and 2 female, aged 21–26. This number of participants is selected to be in-line with other EEG artifact removal works: [9] used 5 subjects, [4] used 12 subjects, [11] used 2 subjects, [16] used 5 subjects, [17] used 8 subjects. Importantly, I want to highlight here that the aim is not to present (or imply to present) a behavioral result where averaging across a larger number of different participants is very significant. The goal is to present the technical results of the artifact removal process, and for this averaging across multiple subjects is not desirable as the artifact is either removed, or not, on an individual record-by-record basis.

All experiments were conducted between 11 am and 1 pm on a weekday to minimize baseline EEG variances and subjects were allowed to take breaks in between experiment runs to prevent fatigue. All procedures used in this study were reviewed and approved by the University of Manchester Research Ethics Committee.

Verification Methods

Time Domain Analysis using descriptive statistics

For the time domain analysis of protocol 1, free-running EEG, in addition to being able to simply see the alpha rhythm (which gives a good visual indication) during tACS stimulation, descriptive statistics of the EEG data collected for sham and tACS stimulation are derived and compared. These methods are a similar technique to that applied to assess the performance of artifact removal in EEG data during fMRI ([4], [11]). Unlike the phantom head data, there is no baseline to correlate the reconstructed data, thus I calculate the complexity, kurtosis, Root-Mean-Squared (RMS) amplitude, and zero-crossings to represent statistical properties of EEG data [18]. These metrics have been selected as: Kurtosis is used in [4], RMS in [11], Complexity is used as a measure of signal entropy which is known to increase in the presence of artifacts [19], and zero-crossings give a time domain estimate of the frequency content which will change if residual artifacts at the stimulation frequency are present.

These statistics are found for each 10 s non-overlapping window of EEG data, with the window size determined from a sensitivity analysis checking the accuracy of each descriptive measure with window size. Although sham and stimulation EEG data are from different recording cases and the statistics are not expected to be identical, the stimulations are deliberately short in duration such that no differences in the brain state are expected to be caused by tACS. The assumption is that the descriptive statistics of EEG data containing residual tACS artifacts will be different from those of sham conditions. This is tested for statistical significance by a 1 way ANOVA ($p < 0.05$) between sham..
and stimulation conditions for each measure for all subjects. Subsequently, a multiple comparisons test using the scheffe’s method [20] is applied to determine the p-values, estimated mean differences and confidence intervals (95% confidence level). Thus if the tACS artifact is correctly removed the ANOVA null hypothesis should be accepted. This is in contrast to the standard ANOVA formulation where the expectation is to reject the null hypothesis. As a result we cannot prove descriptive statistics come from the same population, only that the collected data does not support the presence of residual artifacts which may cause a difference in the descriptive statistics. In isolation the ANOVA results are thus of limited use, but combined with the other verification methods they add weight to the confidence that the tACS artifacts are removed successfully.

**Frequency domain analysis**

Frequency domain analysis is then performed on protocol 1 data by estimating the Power Spectral Density (PSD) of the EEG using Welsh spectrograms. Data is split into 1 s long epochs with a 0.1 s overlap, then each epoch is windowed using a Hamming window. The periodograms (calculated with $N = 2^{15}$) of these epochs are averaged to obtain the PSD estimate. As with the time domain analysis the PSDs between artifact removed EEG+tACS data and the sham are expected to be non-identical, but highly similar due to our short stimulation. To illustrate the potential for individual EEG data responsive stimulations the PSD data is used to extract the Individual Alpha Frequency (IAF) for each protocol (or section of protocol). This is taken as the frequency with the peak PSD value in the alpha range (8–12 Hz), and the ability to extract it during tACS shows how the stimulation frequency could potentially be dynamically adapted to match, or deliberately not match, the underlying brain state.

**ERP reconstruction**

Finally, using the protocol 2 data, ERPs are extracted to show that very low amplitude ($< 10 \mu V$) EEG components are correctly present in the artifact cleaned data, and to demonstrate the potential use of EEG+tACS as part of Brain-Computer Interface systems that are based upon ERPs. To extract responses the data for the visual ERP task is split into 1 s long sections corresponding to the duration for which each stimuli was presented. An ERP detection algorithm was developed to identify successful trials for both face and non-face stimuli by identifying the P100, N170 and P300 peaks. The algorithm searches for the 3 peaks in the EEG data corresponding to each visual stimuli. For a successful trial these peaks are required to be greater than 0.5 times the mean absolute deviation of the baseline EEG data collected during the pre stage of each protocol. If all three peaks are detected to meet the threshold, the ERP is accepted, otherwise it is discarded.

All recorded data was analyzed offline in Matlab (Mathworks, USA). Prior to analysis the data was filtered using 3rd order low pass and high pass Butterworth filters with cut-
offs at 50 and 0.5 Hz respectively.

4.3.3 Results

Time domain analysis

To illustrate the artifact removal procedures, Fig. 4.8 shows the time domain data plotted for one subject for a single trial of the alpha task (protocol 1). Similar to the phantom head results, a burst of alpha activity can be identified after eyes are closed at the 30 second mark, for both the sham and stimulation conditions. This is seen for all three methods of artifact removal, although visually SMA gives a cleaner looking signal with fewer residual artifacts at the stimulation frequency compared to the PCA and AF techniques. This shows that the tACS artifact is removed from individual records as required, it is not an average effect from studying multiple participants.

Figure 4.8: EEG data during the alpha task protocol, at PO8, for a single subject showing sham ([A]) and artifact removed data with tACS at 40 Hz, 1 mA amplitude for the three different methods ([B]-PCA, [C]-SMA and [D]-AF). Eyes are closed at the 30 s mark, after which a small EMG artifact follows. Then bursts of alpha are seen for both sham and stimulation using all three artifact removal approaches. Note that the sham and stimulation are different trial runs and thus the EEG trace for sham and the three other figures are not expected to be identical.

To quantify this artifact removal performance, and summarize the performance across all runs and subjects, Table 4.1 gives the descriptive statistics (complexity, kurtosis, RMS, zero-crossings) of the artifact removed EEG data and EEG data during sham stimulation for data from protocol 1. The ANOVA and multiple comparisons test accept the null hypothesis, which could suggest that no residual artifacts with different descriptive statistics are present. This is further demonstrated by the majority of high p-values (0.9–1) for comparison of sham and stimulation data.
Table 4.1: Mean of EEG descriptive statistics: complexity, kurtosis, RMS amplitude, and zero-crossings; calculated using formulas from [18] across all 5 subjects for all 6 repetitions of protocol 1 (n = 30 for stimulation data and n = 10 for sham data). Also shown are the p-values, estimated mean differences and lower and upper Confidence Intervals (CI) from multiple comparisons of sham data with the three reconstruction methods. One way ANOVA results are also included.

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<th>Estimated mean difference</th>
<th>CI: upper limit</th>
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<td>15.91</td>
<td>0.71</td>
<td>–3.97</td>
<td>2.80</td>
<td>9.57</td>
</tr>
<tr>
<td>SMA</td>
<td>13.66</td>
<td>1.00</td>
<td>–6.90</td>
<td>–0.13</td>
<td>6.64</td>
</tr>
<tr>
<td>AF</td>
<td>15.16</td>
<td>0.97</td>
<td>–5.57</td>
<td>1.22</td>
<td>8.02</td>
</tr>
<tr>
<td><strong>Zero crossings [per 10 s/100]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>12.27</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PCA</td>
<td>13.47</td>
<td>0.93</td>
<td>–2.21</td>
<td>0.70</td>
<td>3.60</td>
</tr>
<tr>
<td>SMA</td>
<td>11.85</td>
<td>0.98</td>
<td>–2.46</td>
<td>0.44</td>
<td>3.35</td>
</tr>
<tr>
<td>AF</td>
<td>12.53</td>
<td>0.97</td>
<td>–2.41</td>
<td>0.51</td>
<td>3.42</td>
</tr>
</tbody>
</table>

**Frequency domain analysis**

The artifact removal performance is illustrated in the frequency domain in Fig. 4.9, which again shows data from a single subject to demonstrate the potential for individually data responsive stimulation protocols. PSD data is shown during 5 Hz, 10 Hz and 40 Hz tACS during the 30 s when the eyes are open, and during the 30 s when the eyes are closed. All of the eyes closed cases show a substantial increase in the alpha band power, as would be expected, in sham data and in true EEG data after tACS artifact removal. This is despite the fact that for 5 and 10 Hz stimulations the stimulation frequency overlaps, or near-overlaps, with the EEG frequency.

In the other frequency bands there is a close correspondence between the EEG powers in the artifact removed data and the sham data, again indicating that there are no residual tACS artifacts introducing distortions at particular frequencies, apart from in the 40 Hz stimulation case. It is important to note the presence of a large peak at 40 Hz for the eyes open case for all methods, but it is lower than the dominant alpha rhythm which allows
Figure 4.9: PSD data, at PO8, during the alpha task protocol for a single subject with stimulation at 5 (top), 10 (middle) and 40 (bottom) Hz, 250 μA amplitude. The protocol is split into 2 sections, Eyes Open (left) and Eyes Closed (right). An increase in alpha activity (8–12 Hz) is seen when the eyes are closed in all cases.

the alpha activity to be seen in the time domain as in Fig. 4.8. Nevertheless this could indicate the presence of a residual artifact in the reconstructed EEG data. This peak is not seen in the eyes closed case (40 Hz stim) when using the SMA technique. The implications of this peak will be discussed in Section 4.3.4.

To demonstrate the extraction of frequency domain information from the artifact removed EEG data Table 4.2 shows the IAFs extracted for a single subject in each stimulation case. The expected increase in IAF is seen when eyes are closed [21] and this change is consistent for both the sham conditions and the majority of the stimulation conditions. It is noted that for this subject the expected increase in IAF is least likely to be seen when using the PCA algorithm, especially during 10 Hz stimulation, where the recorded IAF shifts towards the stimulation frequency.

ERP reconstruction

ERPs can be extracted during the tACS stimulation, as illustrated in Fig. 4.10 when using a 40 Hz, 250 μA stimulation. The ERPs are small and below the free-running EEG noise floor signals, giving strong evidence that neural components are correctly reconstructed after artifact removal.

For space here Fig. 4.10 only shows the average ERPs from one subject and stimulation set up. Similar results are found across all subjects and set ups. The ERP detection accuracy is shown in Table 4.3 where with all different tACS set ups 80% of stimulus presentations result in the detection of a valid evoked response in the EEG. This demonstrates ERP detection even in the presence of tACS stimulation. There is no statistical differ-
Table 4.2: Individual Alpha Frequency during Eyes Open (EO) stage and Eyes Closed (EC) stage for all protocols and artifact removal algorithms.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>EO</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 1</td>
<td>8.03</td>
<td>8.96</td>
</tr>
<tr>
<td>Sham 2</td>
<td>8.07</td>
<td>9.05</td>
</tr>
<tr>
<td>5 Hz, 0.25 mA-PCA</td>
<td>8.03</td>
<td>9.22</td>
</tr>
<tr>
<td>5 Hz, 1 mA-PCA</td>
<td>9.83</td>
<td>9.41</td>
</tr>
<tr>
<td>5 Hz, 0.25 mA-SMA</td>
<td>8.03</td>
<td>9.22</td>
</tr>
<tr>
<td>5 Hz, 1 mA-SMA</td>
<td>8.03</td>
<td>9.38</td>
</tr>
<tr>
<td>5 Hz, 0.25 mA-AF</td>
<td>8.03</td>
<td>9.23</td>
</tr>
<tr>
<td>5 Hz, 1 mA-AF</td>
<td>8.03</td>
<td>9.43</td>
</tr>
<tr>
<td>10 Hz, 0.25 mA-PCA</td>
<td>10.04</td>
<td>9.16</td>
</tr>
<tr>
<td>10 Hz, 1 mA-PCA</td>
<td>10.04</td>
<td>9.37</td>
</tr>
<tr>
<td>10 Hz, 0.25 mA-SMA</td>
<td>8.03</td>
<td>9.05</td>
</tr>
<tr>
<td>10 Hz, 1 mA-SMA</td>
<td>8.03</td>
<td>9.16</td>
</tr>
<tr>
<td>10 Hz, 0.25 mA-AF</td>
<td>8.03</td>
<td>9.08</td>
</tr>
<tr>
<td>10 Hz, 1 mA-AF</td>
<td>8.03</td>
<td>9.08</td>
</tr>
<tr>
<td>40 Hz, 0.25 mA-PCA</td>
<td>9.78</td>
<td>9.70</td>
</tr>
<tr>
<td>40 Hz, 1 mA-PCA</td>
<td>8.03</td>
<td>9.29</td>
</tr>
<tr>
<td>40 Hz, 0.25 mA-SMA</td>
<td>9.19</td>
<td>9.26</td>
</tr>
<tr>
<td>40 Hz, 1 mA-SMA</td>
<td>8.03</td>
<td>9.22</td>
</tr>
<tr>
<td>40 Hz, 0.25 mA-AF</td>
<td>9.40</td>
<td>9.55</td>
</tr>
<tr>
<td>40 Hz, 1 mA-AF</td>
<td>8.03</td>
<td>9.25</td>
</tr>
</tbody>
</table>

ence in detection rates (1 way ANOVA, \( p < 0.05 \)) between the no stimulation sham case and the artifact removed EEG cases, indicating that ERP trials are not lost when tACS is applied. The same number of stimulus presentations allows the same number of ERPs to be detected, important for maintaining the information transfer rate in Brain-Computer Interfaces.

Fig. 4.10 shows a number of expected evoked peaks: P100 (positive peak 100 ms after stimulus presentation); N170 (negative peak 170 ms after stimulus presentation); and P300 (positive peak between 200 and 400 ms after stimulus presentation). It is clear that despite the simultaneous tACS stimulation a wide number of evoked responses can be correctly recorded and detected in the artifact removed EEG data. This is further verified by the form of the results. When a picture of a face is shown a larger N170 response is expected compared to a non-face presentation [12], and this is indeed seen. An average change in N170 depth of 4.3 \( \mu \text{V} \) is seen when a face is presented, demonstrating the ability to detect very small amplitude EEG components in the presence of tACS. Also seen in Fig. 4.10 is the N400 trough, which occurs 300–500 ms post stimuli when the face used is a famous/familiar one [22], as used in this study. An N400 depth of 7.5–8.5 \( \mu \text{V} \) on average was recorded for both sham and stimulation conditions.

Finally, it is noted in Fig. 4.10 that a 40 Hz enveloping is present after artifact removal.
Figure 4.10: Average ERP at PO8 after application of the detection algorithm for sham [A] and stimulation after artifact removal using the three different methods ([B]-PCA, [C]-SMA and [D]-AF), at 40 Hz 250 µA stimulation in a single subject. All peaks are detected at the expected times and the expected increases in N170 and N400 depths are seen when face stimuli are presented. Note that the sham and stimulation are different protocols and thus the EEG trace for sham and the three other figures are not expected to be identical.

Table 4.3: Mean detection scores and standard deviation which represent the percentage of successful trials where an ERP was detected for both face and non-face stimuli. The scores are aggregated for all subjects and all different protocols (n = 30 for all stimulation conditions and sham).

<table>
<thead>
<tr>
<th>Reconstruction method</th>
<th>Mean accuracy</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.84</td>
<td>0.13</td>
</tr>
<tr>
<td>PCA</td>
<td>0.86</td>
<td>0.12</td>
</tr>
<tr>
<td>SMA</td>
<td>0.82</td>
<td>0.15</td>
</tr>
<tr>
<td>AF</td>
<td>0.86</td>
<td>0.15</td>
</tr>
</tbody>
</table>

when using the AF algorithm. This is similar to the 40 Hz peak in the PSD of Fig. 4.9. It is clear that the AF technique leaves a residual tACS artifact in the EEG signal when a 40 Hz stimulation is used. Nevertheless Fig. 4.10 shows that the low amplitude ERP components can still be easily identified despite the presence of the residual artifact.

4.3.4 Discussion

My results provide a systematic overview of the artifact removal process. Successful reconstruction of EEG data after artifact removal has been demonstrated, and verified with no statistically significant differences found when comparing the descriptive statistics of free-running EEG in the time domain. The use of descriptive statistics to quantify tACS artifact removal is novel to this study and as discussed in Section 3.3.5, no other paper presents a quantified analysis to determine the validity of the reconstructed data.
Each of the three artifact removal methods considered here could successfully remove gross tACS artifacts from the simultaneous EEG traces. Using the PCA approach a higher difference in mean (to sham) was seen for the RMS amplitude compared to the other algorithms (Table 4.1), which may be expected due to the use of fewer channels than in [23] in order to allow for a low channel count, out-of-the-lab tACS set up. As discussed in Section 3.3.5, [9] showed that the EEG artifacts of tACS are non-linear and methods such as PCA may not account for this. The changes in impedance of the stimulation electrode cause changes in the output of the stimulator as it is attempting to maintain a constant current. However, both AF and SMA do not assume a constant noise source for the whole duration of the stimulation and both methods account for changes in the source of the stimulation signal where these non-linear artifacts occur [5]. The AF uses the stimulator output as the noise template, this output is regulated by the stimulator to account for changes in impedance caused during the course of the experiment. SMA, on the other hand, adapts to temporal changes in the noise signal by design due to the moving average window that is constantly adjusting the noise template to be removed. The high temporal resolution of both these methods allows for routine adjustment of the artifact template, accommodating for these effects. To my knowledge there is currently very little literature on modelling of the tACS artifact in EEG ([24], [25]) and more research on this would be highly beneficial for improving the artifact removal process further and allowing the introduction of more parametric signal processing techniques.

Both the PCA and AF algorithms had substantial 40 Hz components present after the artifact removal process when stimulating at 40 Hz (Fig. 4.9). Although the presence of such residual artifacts do not prohibit the detection of evoked potentials, it indicates SMA as the preferred artifact removal approach and in everyday usage I believe it gives the best practical performance. These residual artifacts may be a result of distinct changes in electrode impedances that thus result in adjustment of the stimulator output which are further amplified for 40 Hz stimulation as from experience, I found that the Neuroconn DC Stimulator’s output/impedance is more variable at higher frequencies. This effect is not seen when using SMA due to its high temporal resolution as discussed above, however since I set a high forgetting factor (>0.99) for the RLS Adaptive Filter, these residual artifacts are still observed as the filter is adding equal weight to the new and old samples of noise. Thus, in future studies, I aim to implement an on-line measure of impedance that is used to adjust/reset the forgetting factor of the AF as soon as changes in impedance are detected to tackle these residual artifacts.

4.3.5 Summary

Here, I have presented and compared three signal processing algorithms for removing the tACS artifact from simultaneous EEG recordings. The three different methods used (PCA, SMA and AF) were all found to reconstruct valid EEG after artifact removal. The
SMA approach obtained the best overall artifact removal performance. Using all the methods, in the time domain naturally occurring alpha waves could be discerned during tACS stimulation, and in the frequency domain the individual alpha frequency extracted. This shows the potential for using tACS artifact cleaned EEG data in a closed loop manner, adjusting the tACS settings to deliberately match, or not match, the underlying EEG. Both the PCA and Adaptive Filtering approaches were found to leave a residual 40 Hz peak in the EEG traces when stimulating at 40 Hz. Nevertheless using all three methods ERP responses could be detected from a single trial during tACS, with this work being the first demonstration of single trial ERP detection during tACS. This demonstrates the potential for incorporating tACS into future Brain-Computer Interface applications, and for imaging the operation of the brain during tACS in a wide number of cognition and mental health applications. Chapter 6 presents a toolbox that I made as a plug-in to Matlab that enables this by performing tACS artifact removal in real-time while streaming EEG data that is made available for any online assessment.

4.4 Summary

An overview of existing studies that present tACS artifact removal showed a lack of evaluation of the performance of the algorithms, with no quantified verification or comparison to other methods. Furthermore, the previous studies all implemented recording montages with very high channel counts making them unsuitable for out-of-lab applications which is a key requirement for development of therapeutic applications of tACS that utilise real-time imaging information to deliver personalised treatment. Thus, the two methods that I developed (SMA and AF) for artifact removal were designed to be independent of the number of EEG channels used, with the potential for operating in real-time.

The phantom head provides a platform where the baseline activity during stimulation is known and thus the performance of the artifact removal processes can be directly measured using correlation coefficients. The results for the 2 novel methods introduced in Chapter 3 are found to be highly correlated with simulated activity, all above 85% compared to a correlation of approximately 2.7% between simulated EEG activity and tACS-EEG data with no artifact removal. The novel phantom head model also allows for a measure of repeatability when designing and testing any new artifact removal methods in the future and compare their performance with existing ones.

Application of these novel methods on-subject data showed successful reconstruction of both the induced alpha and the evoked N170 ERPs providing verification of the performance of these methods. These findings are further extended, beyond any analysis presented by other studies, using the systematic, multi-stage analysis of features in the time and frequency domain presented here. These allow a quantification to these meth-
ods such that future works can compare (and improve) on this if wanted. Next, in Chapter 5 I show that this level of cleaning is sufficient to allow the determination of different brain states during tACS.

References


Chapter 5

EEG monitoring of working memory during tACS

5.1 Introduction

In chapters 3 and 4, I presented methods for artifact removal that were designed such that they could be implemented offline for analysing pre-recorded EEG during tACS stimulation. These will be extended in Chapter 6 to allow real-time operation, and modification of some of the tACS parameters based upon the current brain activity during tACS.

This chapter investigates applying the created methods in a practical neuroscience task, demonstrating the potential for the use of the new tools. The objective is not to present a new neuroscience result per se, but to show the potential enabled by the new technology created in Chapters 3 and 4. This also provides further verification of the techniques, and provides for the first time, evidence of different brain states being detectable in the EEG during tACS. For doing this, as discussed in Chapter 2, work here focuses on working memory assessment and the use of computer exercises that utilise working memory, to potentially shorted the route to translation for this work as a novel non-pharmacological approach for creating therapies in conditions such as Mild Cognitive Impairment which can be the focus of future work.

Methods for monitoring working memory load using EEG already exist and applications of assessing dynamic features of EEG activity will be discussed in Section 5.2. The experiments for this study were specifically designed to establish the proof of concept that working memory can be monitored during tACS. Section 5.3 presents an experiment where 2 different working memory tasks were carried out during tACS and sham conditions. The results are given in Section 5.4 and have two major implications.

First, the successful separation of the tasks in both sham and stimulation conditions is further verification of the artifact removal process adding to the results presented in Chapters 3 and 4. This task based verification presents a novel way of confirming the artifact
removal process, which has not been considered before. Secondly, presented results validate the use of these techniques to assess working memory during stimulation.

5.2 Why working memory monitoring during tACS

5.2.1 Introduction

Although in the previous chapters I show that it is possible to monitor activity in both the frequency domain (alpha activity) and time domain (face/non-face ERPs), the goal when designing this study was to target a specific application such that techniques presented here can be easily translated to therapy in clinical conditions.

I present an overview of established working memory models in Section 5.2.2, followed by a review in section 5.2.3 of various recent studies that have presented the use of tACS to enhance cognitive function during various working memory tasks. Subsequently, deficits in working memory are known symptoms of various cognitive disorders thereby providing the motivation to focus the presented techniques to monitor working memory. Section 5.2.4 presents a brief overview of studies applying extraction of dynamic features to monitor working memory followed by a summary in section 5.2.5 presenting rationales behind the design of this experiment.

5.2.2 Working memory models

In 1974, Baddely and Hitch [1], extended the previously established unitary Short-Term Memory (STM) model to be the three component model that ‘working memory’ has been defined since. They presented a model that separated a control system (central executive) that processes information from 2 storage components: the phonological loop (sound and language) and the visuospatial sketchpad. The Phonological loop is considered to facilitate language and its capacity is known to be a predictor of ability to learn second languages [2]. Whereas the visuospatial sketchpad is representative of non-verbal intelligence [3],[4]. The central executive was initially defined as general processing capacity but was later extended to comprise of an autonomic system and second process of the Supervisory Activating System (SAS) that could interrupt routine control in case of new stimuli [5]. This three model system did not allow for the phonological loop and the visuospatial sketchpad to interact and thus a fourth component was proposed, the episodic buffer [6], which is controlled by the central executive and allows different systems to be integrated and can be considered the storage component of the central executive [7].

Neuroimaging studies have attempted to localise the cortical locations of the working memory components. The phonological loop has been observed to be located mainly at
the left temporoparietal region [8], [9] whereas the visuospatial sketchpad mainly at the anterior extrastriate occipital cortex [10]. Whereas the executive function is associated with the frontal lobe [11], [12] and is commonly measured using tasks such as the N-Back task (see Section 5.3.2). However there remain points of conflicts with localisation of cortical sources of these components. For example, [13] reported the activation of the dorsolateral prefrontal area when combining two tasks whereas [14], [15] report reduced activity at the same location when combining two other tasks. Furthermore, [7] goes to identify the need to reduce variability in such tasks by mapping the strategy for subjects, which can vary between individuals.

In a review in 2015, [16] present the emergence of state-based models of working memory, which underlie on the principle that attentional selection brings information into the working memory and subsequent prioritisation defines capacity. Further, [16] present 2 categories of state based models, the Long-Term Memory (LTM) models and sensory/sensorimotor recruitment models. The LTM model relies on the fundamental of two different states of STM; the Focus of Attention (FoA) and the activated LTM [17], [18]. The FoA holds information of stimuli which is then transitioned into the activated LTM when attention shifts where the information is processed and stored. The sensorimotor recruitment model’s principle is that attention is allocated to cortical locations [19], [20]. Suggesting that post stimuli, slots are allocated to hold sensory information and the number of available slots defines the STM capacity [21].

5.2.3 Working memory and tACS

In review, [7] states “that working memory underlies the successful execution of complex behaviour, regardless of the cognitive domain or domains being engaged. When working memory fails, so too does the ability to carry out many activities of daily living”. Studies have identified working memory deficits in prevalent neurodegenerative diseases such as Schizophrenia, Alzheimer’s disease, Parkinson’s disease etc, see [22], [23], [24] for reviews of neural synchrony in brain disorders. This has led to a demand for treatment of these symptoms as therapy and/or preventative measures. In particular for early diagnosis and diagnoses of Mild Cognitive Impairment (MCI) where pharmacological treatments are limited to none [25]. For example, at the point of their review in 2015, [25] that to date the only medication available for treatment of mild symptoms in early diagnosis stages is the cholinesterase inhibitor rivastigmine. Even for this, though improvements in cognition and behaviour are exhibited [26], [27] in their review suggest varied clinical responses.

Thus, the need for non-pharmacological interventions targeting cognitive sympotms may play a desirable role as therapeutics. tES is one such method, which as described in Section 2.3 has been identified to have potential to alter cognitive behaviour. Further, the role of oscillatory activity has been documented in EEG/MEG studies during working
memory tasks. Activity in the gamma range plays a role in maintaining information [28] and the number of items to be maintained is known to be directly correlated with the amplitude of activity in the gamma band [29], [30],[31]. Thereby providing a measure of the capacity of the phonological loop and visuospatial sketchpad as described in Section 5.2.2. Alpha activity has been observed to be active during tasks in regions that are irrelevant to ongoing working memory processes [29],[32] with findings supporting the state dependant involvement of different brain regions in working memory processes and thus may be a potential negative measure of the sensorimotor recruitment principle described by [21]. I.e. a decrease in alpha activity during high cognitive load may relate to temporary allocation of the recorded region for slots for short term memory. Theta activity in the hippocampus and prefrontal regions has also been linked to working memory capacity limits [33], [34] and are linked to executive function.

Further, tACS has been shown to alter these neural oscillations and has been used to show enhancement of working memory capacity [35],[36]. In 2014, [35] applied theta tACS on the parietal regions just below sensory thresholds (skin sensations and phosphene perception) on 24 healthy young subjects. Stimulation (compared to sham) was found to significantly increase performance in a visual-array comparison task and the Corsi block-tapping tasks (clicking on square blocks as they lit up on computer screen) that gauge the capacity of the FoA. Also, [36] tested the storage and executive functions described in the episodic buffer and central executive in Baddeley’s multi-component model [1] using tACS stimulated at the frontal and parietal cortex. Stimulation was delivered to 36 healthy volunteers for 15 minutes at theta frequencies with intensity below sensory threshold during three different tasks: forward and backward Corsi block-tapping, forward and backward digit recall and a dual n-Back task (similar to task n-Back described in Section 5.3 but with stimuli of different letters and spatial location of blue squares). A comparison of the storage capacity for three different tasks in sham and stimulation conditions showed positive effect of stimulation in WM storage capacity when stimulating at the left parietal area. These studies confirm the role of the left parietal region on storage capacity and furthermore, the ability of theta tACS to modulate this process. In their study, [37] also tested executive function during a verbal N-Back tasks and found increased working memory accuracy scores after theta tACS (1 mA peak-to-peak) was stimulated at the Dorsolateral Prefrontal Cortex (DLPFC) on 24 female participants. Their findings confirm the role of the left DLPFC with regards to the central executive in multi-component model of working memory and again the ability of tACS to modulate this process. A study investigating the effect of gamma tACS on working memory capacity limits in N-Back tasks found increased performance due to stimulation at higher workloads (3-Back) when stimulating gamma tACS (compared to sham and tDCS) at the DLPFC for 18 healthy subjects [38]. A study also reported increased performance on fluid intelligence tasks after stimulation on the left parietal and also reduced alpha activity after theta stimulation on frontal regions [39]. These recent studies have shown the
potential application of tACS in enhancing working memory performance at both maintaining information and the executive control. These can be thus applied to known cognitive deficits in prevalent neurodegenerative diseases discussed above, to provide therapy that treats symptoms and can potentially delay the progression of these conditions, especially for patients with early diagnoses where there are limited treatments available.

These findings are extended by [40], they stimulated below recorded individual frequencies for 35 healthy subjects while they performed forward/backward digit recall and n-Back tasks (letter based 3-Back). Stimulation was delivered at the parietal region (Pz-FCz montage, 10-20 system) at intensities below sensory thresholds. The ratio between individual gamma to theta oscillations has been shown to present a measure of short term, working memory capacity [41], thus they predicted that by decreasing the individual theta oscillations via tACS, they will increase working memory capacity by affecting this ratio. Their results showed increased working memory capacity due to the customised theta tACS and EEG analysis before and after stimulation showed an amplitude increase in the theta band allowing them to infer modulation of theta oscillations during stimulation but cite that the altered theta frequency returned to original individual values. They suggest that online assessment can further inform their findings but cite the lack of availability of suitable methods for their study design as a restriction.

Therefore, after reviewing existing literature, it is observed that the application of tACS to modulate working memory processes has shown strong potential with studies able to confirm existing models and also modulate ongoing processes with stimulation targeting frequencies of ongoing activity. The potential of using customised tACS stimulation parameters to provide individual therapy for modulation of working memory capacity was shown by [40] and furthermore, they even go on to highlight the benefit of adding online EEG assessment to their study design to further inform their findings. Also, EEG is commonly used to assess working memory and recent studies have presented the use of dynamic/non-linear features such as Fourier and Wavelet transforms to determine working memory load [42]. Real-time assessment of working memory can have key implications when considering decision making during critical tasks, especially for individuals in high stress environments such as the military and emergency medical care.

5.2.4 Summary

A review of existing models of working memory and their role in mental health presents a clear opportunity for interventional therapy using tACS. Techniques to monitor working memory using EEG during tasks already exists and the ability to monitor EEG during stimulation opens multiple avenues for research in both study of working memory models and application of tACS to enhance performance. Additionally, dynamic measurements of EEG data have been used for assessment and diagnosis in Alzheimer’s Disease ([43]), depression [44], attention deficit/hyperactivity disorder [45] and autism [46].
Also, working memory deficit is a known symptom of MCI ([47],[48] and [49]) which often leads to dementia and, there remains limited availability of treatments for various cognitive disorders [25]. Thus evaluation of neurological activity during high cognitive load can provide insight of the pathology of these disorders and allow for application of non-pharmacological treatments.

Using techniques described in Chapter 3, it is now possible to monitor EEG during tACS for the first time. These new neuroimaging methods allow for deeper understanding of the effect of tACS and can also allow studies to test hypotheses on the different models of working memory by exploring the effect of stimulation on the neural oscillations of the involved regions. Further, this also opens the possibility of BCIs which modulate tACS parameters based on ongoing neural activity. Such a system has multiple applications in previously mentioned cases of performance monitoring and maintenance in crucial tasks in high stress environments and for MCI/early-diagnosis patients for different forms of cognitive impairments. The addition of tACS as an interventional tool presents development of a truly personalised therapeutic treatment in mental health which can potentially delay/reverse the progression of these conditions.

The goal of this experiment was to present the ability to use EEG for monitoring workload during tACS for the first time. Further, recent publications present an increasing demand for such methodologies to further inform findings and to develop experimental protocol for more targeted therapy. Based on the the review presented in this section it was decided to monitor EEG during two different working memory tasks. The backwards digit recall was chosen as it requires components of both verbal short term capacity and executive function, and a visual n-Back task was also chosen such that it also requires processing by the executive function like the digit recall task but employs the use of visual working memory capacity. Both these tasks activate the central executive but use different forms of working memory capacity (phonological and visuospatial sketchpad) such that the ability to successfully separate the classification of these tasks using dynamic features will provide a strong proof of concept of EEG monitoring of workload during tACS stimulation.

### 5.3 Methods

#### 5.3.1 Objectives

Subjects were asked to perform two working memory tasks, nBack and backwards digit recall. As discussed in Section 5.2, both these tasks utilise the central executive of the multi-modal working memory model but use different forms of working memory capacity and are thus ideal to pair in this study.
The goal of this experiment is to show successful separation of data during different tasks in both stimulation and sham conditions using a machine learning classifier with features derived from discrete wavelet transforms of the data in different frequency bands. After an initial training session, the experiment was split into 3 sessions: sham, stimulation and control. Since no significant difference in performance between SMA and AF were observed in previous results, see Sections 4.2 and 4.3, only SMA was applied to stimulation data as it did not require the signal output and thus the methods are more repeatable for other groups without requiring specific hardware.

The successful separation of both the control and task sessions during stimulation will confirm absence of stimulation artifact after reconstruction, whereas the successful separation of the sham and stimulation conditions will represent an effect of stimulation on neural oscillations. Finally, successful separation of data from the two different tasks in both sham and stimulation conditions will provide evidence of working memory monitoring during tACS.

5.3.2 Participants

There were a total of 10 subjects, five male and five female, aged 20-35 years. All experiments were conducted between 2-5 pm on a weekday to minimise baseline EEG variances and subjects were allowed to take breaks in between protocols to prevent fatigue. All procedures used in this study were reviewed and approved by the University of Manchester Research Ethics Committee.

5.3.3 Working Memory Tasks

Subjects were asked to perform two working memory tasks:

- Visual nBack - tests visual working memory span and executive function, the subject is presented a list of visual stimuli and asked to recall if the current stimuli matches the $n^{th}$ stimuli before the current stimuli. That is if the current stimuli matches the stimuli 2 iterations (2-back) or 3 iterations (3-back) before and so on (see Fig. 4.10). The images used are of tasks and activities easily identifiable to the participants. 5.1

- Backward digit recall - tests verbal memory span and executive function. A list of numbers presented to the subject, who is then asked to recall them in reverse order. Numbers are displayed one at a time and each for 1 second, after which the subject is asked to enter them in reverse order of display. The subjects are asked to do as many repetitions of this as they can in 2 minutes.
5.3.4 Practice Session

Before the actual experiment subjects were invited to a practice session, during which they performed the above mentioned tasks multiple times at different difficulty levels. The purpose was to familiarise the subjects with the tasks and also to establish a baseline of difficulty at which each individual's performance peaks (n for the nBack task and the span for the digit recall task). This minimises the influence any learning effects that repetition may have in the result of the study.
5.3.5 Experimental Protocols

The experiment was split into various experimental sub-protocols each 4 minutes long. These were further divided into three stages ranging from 1-2 minutes with the procedure:

1. Pre: One minute of background EEG recording;
2. During: 2 minutes; task performed, EEG recorded and tES stimulation/Sham;
3. Post: One minute of background EEG recording.

This closely matches the experimental protocol in Chapter 4, as similar to that experiment, the goal here is to not produce behavioural effects but provide a proof of concept for monitoring working memory during tACS. Thus a minimal dosage of stimulation is applied. There was a break after each 4 minute protocol and the participant would be asked when they were ready for the next protocol to prevent fatigue. They perform both tasks at the difficulty level predetermined at the practice session. Each task was repeated 3 times, thus resulting in a total of 6 tasks, each 4 minutes long.

5.3.6 Experiment Sessions

After the practice session, each subject was asked to come in for 2 experiment sessions. The total run time of each experiment was roughly 30 minutes long (6 protocols, including the breaks between tasks). It also took up to 20 minutes to set up the EEG and tACS electrodes. Thus subjects were asked to expect the experiment session to last on average an hour.

The experimental protocols were identical for both the sessions with the exception that tACS was applied in one session and the other session was the sham session where no tACS is applied (but the tACS electrodes were still placed to ensure the participants are uninformed). Each experiment session was separated by the other by at least a period of 24 hours and at maximum 1 week.

5.3.7 Control Session

All subjects were also asked to do a control session which consisted of a 5 minute tACS stimulation, using the same montage as for other protocols, where the subjects were asked to relax, keep their eyes open and not do anything for the duration, essentially the same as Pre & Post sections of the protocols. This provided a baseline for the stimulation condition to compare with data collected during stimulation while doing tasks.
5.3.8 EEG and tACS Setup

An 8 channel EEG montage was used for this study with the ground and reference channel placed at the Cz. The 8 electrode positions were determined by using the ten–twenty system: Fp1, F3, F4, C3, C4, P4, O1, O2. All electrode impedances were below 10 kΩ and the data was sampled at 500 Hz. EEG was recorded using the Enobio (Neuroelectrics, Spain).

Stimulation was delivered using the DC-Stimulator PLUS (Neuroconn, Germany). The tACS electrodes were placed at the Fp2 and P3 which are reflective of montages that target working memory effects of tES. tACS was stimulated at 1 mA peak-to-peak at 5 Hz for the duration of the working memory tasks (2 minutes) with a ramp up and down time of 1 seconds each. These parameters were selected to mimic previously used montages in experiments looking at effects of parietal stimulation in working memory [35], [36].

5.3.9 Data Analysis

Fig. 5.2 shows the data analysis process, each step is discussed in detail later in this section. Recorded EEG data is filtered and then if tACS stimulation was applied, the tACS artifact is removed using Superposition of Moving Averages (SMA) as described in Section 3.4. Next, an EEG feature matrix is extracted consisting of power and entropy measures for the gamma, beta, alpha, and theta frequency components calculated using Discrete Wavelet Transforms (DWTs). Subsequently, machine learning classifiers are used to train.

Pre-processing

All recorded data was analyzed offline in Matlab (Mathworks, USA). Prior to analysis the data was filtered using 3rd order low pass and high pass Butterworth filters with cutoffs at 45 and 0.5 Hz respectively.

tACS Artifact Removal

SMA was applied unmodified from the formulation described in Section 3.4.

Feature Extraction

An EEG feature is a unique measurement/representation of a segment of data which reflect evoked or ongoing neural activity. The Discrete Wavelet Transform (DWT) allows time-frequency localisation of a signal allowing representation of the data in fixed
Figure 5.2: Data analysis summary from the point of recording to classification of extracted features.

‘wavelets’ which provide dynamic and temporal features of the data [50]. The DWT has a higher resolution at lower frequencies which are most dominant during cognitive processes, making it ideally suited for use with EEG data. It can be thought of an extension of the Fourier Transform allowing for signals which are non-stationary. A stationary signal does not vary with time in the frequency domain whereas a non-stationary signal is time dependant in the frequency domain. The Fourier Transform assumes this when calculating Fast Fourier Transform (FFT) of EEG data, it is calculated for certain time windows limiting its resolution in the time domain whereas the DWT does not assume a stationary signal and has a higher resolution in the time domain but is limited to a lower frequency resolution. However for EEG analysis, the active frequency bands of neural oscillations are wide and allow for use of DWTs even with a lowered frequency domain resolution. Thus it is widely used in EEG data analysis [51]–[55].

The EEG signal for each channel was split into non-overlapping windows of 5 seconds. Each window is then downsampled to 256 Hz so that the frequency bands of the components of the DWT better match the cognitive frequency bands: theta (4-8 Hz), alpha (8-16 Hz), beta (16-32 Hz) and gamma (32-64 Hz). The DWT coefficients are then determined for each component using the ‘db1’ wavelet basis function. These coefficients are then used to reconstruct the signal for each component such that it only represents
Figure 5.3: The EEG signal after filtering and artifact removal (for stimulation cases) is downsampled to 256 Hz. DWT coefficients are then determined for each frequency band. Finally the signal is reconstructed using the coefficients to provide the DWT signal that gives a time-frequency localisation of for each particular frequency components.

the particular frequency band (see Fig. 5.3). This signal is full rate (256 Hz) representation of the frequency content in that decomposition scale over time.

Power and Shannon entropy measures for four of the DWT signals (representing the gamma, beta, alpha and theta frequency bands) are then calculated for each 5 second window and used as features. This is repeated for all 8 channels, thus a feature matrix of 64 features is established.

Classification

There were two different models used for classification, a 6-class and a 4-class model. The 4-class model was used to show successful separation of data while performing task from data while not performing the task (for both sham and stimulation) and also separate sham and stimulation data while performing the task. This, as explained in Section 5.3.1, will confirm successful artifact removal and also show effect of stimulation in the neural oscillations. The 6-class model will be used to show successful separation of the two different task showing successful monitoring of working memory during tACS.

The classes for the nBack and the Digit Recall data were built separately with each of the 4 classes divided as follows (See Fig. 5.4):
Figure 5.4: Experimental protocol representing 4 classes used as input to the classifier for the 4 class model. The ‘Baseline’ class is the Pre & Post sections of the protocol, the ‘Task’ class is data from when the subject is performing the task without stimulation. In the 4-class model ‘Task + Stim’ represents the subject performing the task in the stimulation experimental session and the ‘Baseline + Stim’ is stimulation while no task is performed. For the 6 class model the ‘Task + Stim’ and the ‘Task’ condition are further split into 4 classes, which represent the two different tasks (nBack and Digit Recall) with and without stimulation.

1. Baseline : The Pre & Post section of each protocol.
2. Baseline + Stim : The control session.
3. Task : Task (n-Back or digit recall) without stimulation.
4. Task + Stim : Task (n-Back or digit recall) with stimulation.

For the 6-class model, the ‘Task’ and ‘Task + Stim’ classes were replaced by each of the two task conditions, with and without stimulation, thus the classes were:

1. Baseline : The Pre & Post section of each protocol.
2. Baseline + Stim : The control session.
3. nBBack : Task without stimulation.
4. nBack + Stim : Task with stimulation.
5. Digit Recall : Task without stimulation.
In the 6-class model, data from both tasks was used to build a single model such that the classifier can differentiate between two different measures of memory span (nBack and digit recall).

The data was classified using the built-in function in matlab for the Linear Discriminant Analysis (LDA) classifier based on Fisher’s discriminant analysis [56]. It assumes that the data each class is based on different Gaussian distributions with the same covariance and aims to express each dependant variable (the classes) as a linear combination of the features. It was extended to use for multiclass models by [57].

Since the data sample sizes are not equal (different number of epochs for each class), the chance level of the model cannot be assumed to be \(1/(\text{number of classes})\), since there is not an equal chance that an epoch can be assigned to any class if the classifier chose the output at random.

Thus chance level was calculated for each model by measuring the performance between the test data and a randomly sorted output of classifier 10000 times. The average performance measure between the true class of the test data and the randomly sorted classifier output was estimated to be the chance level for each model. The calculated chance levels are included with the results in Fig. 5.5.

5.3.10 Classification validity

An assumption for supervised learning using classifiers is that the data is Independent and Identically Distributed (iid) [58]. This implies that the order of the data is not relevant and thus all epochs are from the same arbitrary distribution [59]. In practice this means that the order in which the data (each epoch) is sorted for input to the classifier should not effect the performance of the classification. This is not automatically satisfied for time series data such as the EEG which evolves over time, where samples collected close together in time may be more similar than those collected further apart in time [60].

To investigate this, data for each subject, for each 5 second window and for each class were used to build a feature matrix. To test that the collected data is iid, before training and testing the classifier, the data matrix is randomised to mix the time order of the collected features. Then 65% of the data used to train the classifier and the remaining 35% is used for testing the performance of the classifier, this process is repeated 1000 times. The randomised sorting of the features of the data will result in different varying performance of the classifier if the data was non-iid. After the 1000 repetitions for each subject, no differences in the performance of the classifier were observed, thereby confirming that the collected dataset is sufficiently iid for the purposes of this study. The result of this test are included in Fig. 5.5, Fig. 5.6 and Fig. 5.7 as the error bars.
5.4 Results & Discussion

5.4.1 4-class model

Fig. 5.5 shows the performance of the classification models. The 4-class model for both tasks performs with accuracies above 80% (and above chance level), showing that during both tasks EEG data from each class is different between sham and stimulation and also that the data from the control or baseline classes is different for sham and stimulation as well.

The successful separation of both the tasks during stimulation and sham conditions shows an effect of stimulation is observed in the data, but to validate this, it needs to be confirmed that this is not due the presence of residual artifacts. This is shown by the successful separation of the control and task data during stimulation. If there were residual artifacts present in the data, the classifier would not be able to separate the control and task epochs during stimulation as they would dominate the recorded signals.

To show that the performance of the 4-class classifier is not biased by any particular class, Fig.5.6 shows the percentage of epochs classified correctly for each class for both the n-Back and the digit recall cases. All classes perform similar to each other and the data is seen to be not biased by one class outperforming the rest.

The results present a novel method to verify the accuracy of artifact removal using machine learning classifiers and the separation of the task in sham and stimulation conditions suggests that the features extracted from the EEG data are affected by stimulation.
inferring an effect of tACS on ongoing neural activity, though further investigation is required to identify the characteristics of this effect. This may be achieved by a combination of techniques such as identifying the most dominant features used by the classifier ([61], [62]) or the use of computational models of the neural pathways active during the memory tasks ([63], [64], [65]). This is not the aim here, which was to provide the tools to do such investigations in the future.

### 5.4.2 6-class model

The 6-class per–subject model also has a considerably higher performance than chance level. Not only is data with and without stimulation successfully classified but additionally, the classifier is able to separate the nBack and digit recall tasks, with and without stimulation, from the same model. This advocates that the methodology applied in this study can be successfully used to monitor workload during tACS stimulation as evidenced by successful separation between the two different measures of memory span applied here.
Table 5.1: Performance and standard deviation of subject independent classifier for each subject.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Performance</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.631</td>
<td>0.0134</td>
</tr>
<tr>
<td>2</td>
<td>0.672</td>
<td>0.0156</td>
</tr>
<tr>
<td>3</td>
<td>0.722</td>
<td>0.0139</td>
</tr>
<tr>
<td>4</td>
<td>0.685</td>
<td>0.0152</td>
</tr>
<tr>
<td>5</td>
<td>0.692</td>
<td>0.0113</td>
</tr>
<tr>
<td>6</td>
<td>0.683</td>
<td>0.0126</td>
</tr>
<tr>
<td>7</td>
<td>0.611</td>
<td>0.0144</td>
</tr>
<tr>
<td>8</td>
<td>0.697</td>
<td>0.0108</td>
</tr>
<tr>
<td>9</td>
<td>0.670</td>
<td>0.0125</td>
</tr>
<tr>
<td>10</td>
<td>0.707</td>
<td>0.0125</td>
</tr>
<tr>
<td>Mean</td>
<td>0.677</td>
<td>0.0133</td>
</tr>
<tr>
<td>Chance Level</td>
<td>0.182</td>
<td>0.0150</td>
</tr>
</tbody>
</table>

Fig. 5.7 shows that, similar to the 4-class model, the performance of neither of the six classes bias the performance of the classifier with similar percentages of epochs classified correctly for all classes in the 6-class model.

5.4.3 Subject independent classifier

The three models discussed so far have been applied individually to data for each subject and present a system that is capable of monitoring workload during tACS stimulation. However, since the models thus far have only been applied to subjects individually, they require training each time when used with new subjects.

To create a subject independent classifier, data from the feature matrices of all subjects was normalised by dividing each feature observation with the absolute maximum of said feature for each subject. These normalised features were then concatenated to create a single matrix of normalised features from all subjects. Subsequently, 50% of this data was selected at random to train a classifier which was then used to classify normalised data for all subject separately, this was repeated 1000 times.

As seen in Table 5.1, the performance for all the subjects using the inter–subject classifier is 67.7%, which though does not perform as well as the model subject dependant model, is still over 3 times the chance level of 18.2%. Thus now I have presented a subject independent classifier able to monitor workload during tACS stimulation with performance substantially higher than chance level. It should be noted that the average performance using this model is lower than the mean performance using the subject dependant models.
5.5 Summary

Although as discussed in chapter 3, previous studies have presented the removal of tACS artifact from EEG, this is the first study to present monitoring of dynamic features during tACS post artifact removal. I present methodology that further validates successful tACS artifact removal from EEG using the developed technique. Accurate classification of data was observed between the baseline and task conditions during both stimulation and sham confirms that the successful separation by the classifier is based on EEG differences between the different cognitive states. Also allowing workload monitoring during stimulation opens the door for the design of BCIs that can incorporate EEG measures of cognitive load to deliver personalised tACS.

We were successfully able to separate two different tasks that measure different forms of working memory capacity (nBack and digit recall) during tACS and further, the inter-subject classifier was adapted to run for any subject without requiring prior training on a particular person. Even though the subject independence comes at a trade-off of slightly lower performance, the results are significantly above chance levels. This common classifier is the first step towards building a robust interface that can provide closed-loop therapy to subjects that is easy to setup in out-of-lab environments.

The next step is to use the same measures to provide a quantitative measure of the workload during tACS which can provide feedback to the stimulator to build a closed-loop stimulation BCI which can be used to provide personalised cognitive treatment to individuals based on their response to stimulation.

References


Chapter 6

tACS-EEG toolbox

6.1 Introduction

The goal of this thesis was to build methods for closed-loop tACS stimulation using EEG feedback. The first key milestone to achieve this was identified to be the designing of methods that allow tACS artifact removal. As discussed in Chapter 3, due to the presence of artifacts, majority of studies to date have focused on comparing changes in activity before and after stimulation using techniques such as EEG, fMRI and MEG but the need for identifying activity during stimulation was identified. The tACS artifact removal algorithms presented in Chapter 3, and comprehensively validated in chapters 4 and 5, now provide us with methods that can enable the development of the closed-loop experimental paradigms.

This chapter presents the development of a toolbox that records EEG data during tACS in real-time with an interface capable of stimulating in closed-loop. Section 6.2 discusses the objectives that were incorporated when designing the toolbox. Section 6.3 presents an overview of the toolbox design, detailing the structure for collecting and passing EEG data, and the options available for providing closed-loop stimulations based upon these. Section 6.4 presents how the algorithms are modified from offline to online use, with Section 6.5 demonstrating the operation and performance of the toolbox, showing that it is suitable for real-time use with a negligible loss in cleaning performance compared to the offline algorithms. Finally, Section 6.6 is a summary of the detailed toolbox presented in this chapter.

6.2 Overview

Following the development of tACS artifact removal and parallel developments in modelling and computational techniques [1] have opened the door for targeting stimulation based on ongoing EEG activity. Thus recent developments in the field have presented the benefit of developing true closed-loop techniques and expanding existing EEG-triggered stimulation protocols.
However, the tACS algorithms developed so far have been designed and used only \textit{offline}. That is, applied to pre-recorded data only, after the experimental protocol has been completed. This is a highly useful tool in its own right, but passes the question whether the tACS artifact removal could be performed \textit{online}, in real-time to the collected EEG data as it is collected. Furthermore it is necessary to develop techniques that assess the artifact corrected data in real time, for example the processing of the classifier presented in Chapter 5 which is able to monitor workload during tACS in real-time. Subsequently algorithms and interfaces that allow the tACS stimulation parameters to be adjusted based on these real time assessments. Thus, there are certain technical development steps required before closed-loop protocols can be applied for neuromodulation techniques:

- Real-time tACS artifact removal;
- Online assessment of EEG activity during tACS;
- Hardware and algorithms to adjust tACS parameters in real time.

Manufacturers like Neuroconn and Neuroelectrics offer real-time tACS artifact removal with the Neuroprax and Starstim devices respectively. However due to the extra cost and exclusivity to the respective stimulators and EEG devices, researchers have been slow to adopt these methodologies. Further, there is little to no verification available to these techniques in existing literature as discussed in Chapter 3. In addition to the offline algorithms from Chapter 3, there is the opportunity to contribute online algorithms in an open source toolbox which can be integrated with any EEG system, and extended/adapted by any particular user/group to allow groups to design protocols for closed-loop stimulation regardless of their equipment and also to provide them access to verified algorithms at no extra costs.

In this chapter I present the \textit{tACS/EEG toolbox} I developed as a plug-in for Matlab (Mathworks) that allows real-time tACS artifact removal using the SMA and AF techniques presented in Chapter 3 that were adapted to operate in real-time. Algorithms were also designed to monitor alpha activity and other phase/frequency measures during stimulation.

This toolbox is still under development. As will be discussed in Section 6.3.5, there are certain hardware restrictions which prevent the testing of the closed-loop operation. However, the toolbox is shown to be capable of monitoring and assessing EEG activity during tACS and is in principle capable of closed-loop stimulation though the testing of this operation remains to be completed.
6.3 Toolbox Design

6.3.1 Introduction

The goal of this toolbox is to allow researchers access to EEG data during tACS stimulation while running various cognitive tasks and be able to apply closed-loop adjustment of the tACS stimulation parameters based on the ongoing EEG activity. Fig. 6.1 represents the architecture of the toolbox function.

Section 6.3.2 describes the control of the tACS stimulator using the toolbox. The toolbox is currently designed and tested to stream EEG data to Matlab using the Enobio (NeuroElectrics) and the Truscan (Deymed, provided by Rogue Resolutions Ltd.) the function of which is described in Section 6.3.3, however the goal is to keep building a library of devices that are able to stream data to Matlab for use in the toolbox. The filtered and artifact removed data is then passed through an online assessment algorithm which will be determined based on the current cognitive task as described in Section 6.3.4. The final operation of the toolbox is the use of the assessment of the cognitive tasks to adjust the tACS stimulation parameters, thus providing a closed-loop system that can be used to deliver custom therapy which optimises the stimulation, and thereby the treatment, individually for subjects. These adjustments can vary from changing stimulation intensity, frequency or phase to achieve the desired effects and are described in Section 6.3.5. The overall Graphic User Interface (GUI) of the toolbox is shown in 6.2. The operation of the toolbox requires the following equipment and software:
This toolbox presents a common tool that researchers can use to conduct studies exploring the effects of tACS during stimulation and furthermore, allow them to design and test closed-loop paradigms to provide truly personalised therapy for mental health in conditions where at times there are no non-pharmaceutical treatments available. Additionally, by continually adding to the library of compatible hardware, cognitive tasks and close-loop algorithms; researchers can easily repeat protocols from other groups. This is a positive step towards addressing the conflict in the field when studies are repeated by different labs. It will allow researchers to control some of the variables (like stimulation conditions and cognitive tasks) and thus begin to either provide more repeatable results or narrow down the cause of the observed variance.

6.3.2 tACS control

The initial stimulation parameters need to be provided as input to the Graphic User Interface (GUI), the user is required to enter the stimulation frequency, amplitude, the duration of stimulation and the duration EEG data to be collected before and after stimulation; see Fig. 6.2. The user is also asked to select the method of artifact removal (SMA or AF). For viewing cleaned data in real time SMA or AF can be used. The toolbox is still under development and currently only the AF has been tested for closed-loop modes.
Due to availability and it being the most commonly used tES device, the toolbox currently is only designed to operate using the Neuroconn DC plus stimulator. For viewing data only with a fixed set of stimulation parameters, these parameters must be set in the Neuroconn hardware (using it’s front panel display) and the toolbox then just starts/stops the stimulation using the neuroconn trigger input at the correct point in the wanted experiment. In closed-loop operation, the toolbox generates the whole stimulation signal and uses the DAQ to send an analogue signal to the remote input of the Neuroconn which can be modulated based on the online assessment.

### 6.3.3 EEG Stream

Currently, due to availability, the toolbox is designed to function using the Enobio and the Truescan devices and EEG data is streamed in real-time to Matlab using TCP/IP and UDP streams, respectively for the two devices. As shown in Fig. 6.2, users are required to enter filter properties for the EEG display and currently the toolbox is designed to display the stream for a single, user selected, channel.

For the Enobio, data is sampled at 500 Hz and streamed in packets of 200 samples (0.4 seconds). High-pass and Low-pass filters are applied to each packet and if tACS stimulation is on, the artifact removal algorithms are also applied to each packet. The Truscan, in its Matlab stream, samples the data at 200 Hz, and streams data in packets of 16 samples (0.08 s). For consistency, the toolbox waits to collect 0.4 s of data (25 packets) before applying filters and tACS artifact removal (if stimulation is on). Fig. 6.3 displays an example stream of EEG data with and without artifact removal at a single channel.
6.3.4 Cognitive tasks and Online Assessment

The online assessment of stimulation is entirely dependant on the cognitive task and stimulation application being explored during each particular protocol. This is the most expandable section of the toolbox as each research group will have their unique cognitive tasks, each of which will require custom algorithms for evaluating the subsequent data.

Over the course of my research, I have designed and implemented the following tasks that are operated in Matlab:

1. Alpha Induction - Eyes open/close - as described in Section 4.3
2. SSVEPs using LED goggles - similar to phosphene task described in Section 2.3
3. Face/non-face ERP task - - as described in Section 4.3
4. Digit Recall - Forward and backward - as described in Section 5.3
5. nBACK - sound/shape - as described in Section 5.3
6. Dual nBack - sound and shape - similar to described in Section 5.3

For each of these tasks there are multiple methods of assessing the ongoing activity as well. The toolbox can extract information from the EEG trace to determine the state of the user and/or what form for closed loop adaptation should be applied to the tACS. For example, the WM classifier from Chapter 5 can be adapted to determine whether the user is in a high or low workload state and thus adapting the stimulation to potentially affect this. The goal of this study is to provide proof of concept and for that I implemented Alpha monitoring during stimulation, however the toolbox can be expanded to incorporate all the above tasks and many more as required.

As seen in Fig 6.4, for epochs of 2 second, the peak alpha frequency and its power are measured and plotted in a real time. This data was collected using the phantom head model described in chapter 3. For the simulated data, the subject closes their eyes the 30s mark and the increase in alpha activity is easily identified even under stimulation.

6.3.5 Closed-loop operation

For the closed-loop mode, the DAQ is used to send the stimulation signal to the remote input of the Neuroconn DC Stimulator such that the voltage signal input is mirrored by the current generator of the Neuroconn. However, in practice, the remote input terminal is not isolated from the current generator and thus any DC offsets introduced from the DAQ will also be mirrored by the stimulator. The solution is to use an isolated DAQ.
but one was not available. The designed operation for the closed-loop stimulation is described below however testing of these protocols remains to be conducted.

The most basic operation of the closed-loop system is to adjust the stimulation amplitude based on ongoing assessment. This is implemented using the alpha band power monitoring. A threshold of Alpha power was set at which the stimulation amplitude is increased or decreased based on protocol design. The next stage is to stimulate at individual active frequency based on ongoing activity. The current design of the interface is set to achieve this by recording, for epochs of 2 s each, the peak alpha frequency (8-12 Hz). If the measured frequency is 0.1 Hz greater or less than the previous measure of alpha power, then stimulation frequency for the next epoch of 2 s is changed to the measured individual alpha frequency.

The final step in constructing closed-loop techniques is the design phase matching the stimulation waveform with ongoing EEG activity. This will be tested by assessing the EEG activity after every 2 seconds. The phase difference between the recorded EEG data (at the desired frequency) and the stimulated tACS signal will be calculated. This phase difference will then be applied to the stimulation waveform for the next epoch. Though it should be noted that this method requires optimisation. By the time the next epoch of stimulation is generated, time passes and thus the applied phase difference is behind the ongoing activity. The plan to overcome this delay is to include a timer in this operation and then based on the measured delay, the outgoing stimulation will be shifted to match the ‘predicted’ phase of EEG activity at time of the next stimulation.

The design and code for this interface is available and once the appropriate DAQ or isolator for the DAQ signal is made available testing will begin.
6.3.6 Summary

The presented toolbox is capable of real-time artifact removal of EEG activity during tACS. It is designed to use a TCP/IP or a UDP stream to transmit data from an EEG device to Matlab (Mathworks Inc.) and is thus, in theory, compatible with any EEG device that is capable of streaming data via the two methods. This is showcased by using the Enobio (TCP/IP) and the TruScan (UDP) devices.

The algorithm is capable of monitoring alpha power and IAF (or frequency power and individual frequencies in any band in theory) and is designed to be able to adjust stimulation frequency and power based on these assessments. Furthermore, the alpha power and ERP tasks are incorporated into the toolbox such that these tasks can be operated in synchrony with the EEG stream with artifact removed data available for assessment to adjusting stimulation. The software interface for testing closed-loop stimulation has been designed however hardware restrictions meant that the testing of this setup could not be performed to include here.

6.4 Real-time artifact removal algorithms

6.4.1 Introduction

The different available algorithms were considered to be added into the toolbox. PCA is particularly poor for this as its accuracy and performance depend on the size of the input matrix, and it gains very much from having a longer duration of data collected before analysis. When comparing SMA and AF, a number of factors are relevant. Both can operate on just one channel of EEG data, and scale naturally to the number of channels used. Our implementations on a desktop computer with an Intel i7 processor and 16 GB of RAM take 0.01 s and 0.04 s for SMA and AF respectively to process 1 s of EEG data.

6.4.2 SMA

As described in Section 3.4, SMA applies a sliding moving average to segments of EEG data which are averaged across the current segment as the central epoch and its neighbouring epochs. This includes both past and future epochs, which is not possible to implement in real-time. Thus for the toolbox, SMA was adapted to average the current window and the previous 10 epochs.

Thus SMA has a requirement on the minimum length of data needed before the reconstructed data is accurate. As 10 epochs of tACS artifacts are averaged to produce an artifact template, sufficient time must pass to allow these 10 epochs to occur. As shown
in Fig. 6.5, this enforces an extra convergence time of 10 periods of the stimulation frequency (each epoch = period of stimulation frequency) along with the existing convergence caused due to the ramping up of the stimulation amplitude as well. At the end of stimulation an artifact is also observed, this is due to the ramping down of the stimulation amplitude. This in unavoidable due to the way in which the SMA template is generated. It means the online removal performance is expected to be worse, but principally at the start/end of the stimulation. During the main stimulation time good performance can still be obtained.

6.4.3 AF

The toolbox implements a Matlab filter object to run the RLS adaptive filter described in Section 3.4 in real-time. The artifact amplitude is estimated by calculating the RMS of the recorded EEG data every 400 ms and the output signal from the stimulator is adjusted based on this estimate. The adjusted noise and recorded EEG are then used as inputs for the AF with clean EEG as the output.

The AF has a small convergence at the start of stimulation, this will be a factor of the filter order and similar to SMA, an artifact is seen at the end of stimulation due to the ramping down of the tACS amplitude, see Fig. 6.5.
6.4.4 Summary

The toolbox is capable of implementing both the SMA and AF algorithms in real-time. The AF algorithm does have the hardware limitation of the tES hardware requiring the stimulator output signal but provides a shorter convergence time which potentially can decrease the latency of closed-loop operations. Both these algorithms were designed with real-time, channel independent operation and are thus ideal for use with the toolbox with a focus towards out-of-lab mobile setups.

6.5 Toolbox Performance

6.5.1 Introduction

Section 6.3 presented modifications to the created artifact removal algorithms to allow them to operate in real-time. It is thus now necessary to re-verify the algorithm performance and to take into account the effects of amplifier latency, computer performance issues and similar, which may mean that the practical application of the artifact removal for the toolbox does not obtain exactly the same performance as the offline algorithms presented in Chapter 4.

Three major criteria were considered when assessing the performance of the toolbox. The latency of each packet of streamed data. The throughput of the system, for this case defined as the ability of the toolbox to maintain the stream/latency. Finally, I assessed the performance of the real-time artifact removal using the modified algorithms.

Section 6.5.2 described the experiment setup methods used for testing. The latency and throughput of the toolbox are presented in Sections 6.5.3 and 6.5.4 respectively and the real-time artifact removal results are presented in Section 6.5.5 with a brief summary of performance in Section 6.5.6.

6.6 Methods

The phantom head model introduced in Chapter 4 was also a critical tool in both designing and testing the toolbox. Similar to the artifact removal verification, it allowed for a baseline EEG to be measured in a controlled environment to test the latency and throughput of the EEG stream. It means that one computer generates both the EEG signal (to be inputted to the model), and the cleaned EEG data so they are fully in sync and precise timing measurements are possible. Subsequently, the phantom head model was also used to test the accuracy of the real-time artifact removal algorithms.

All measurements for latency and throughput were recorded using the lab laptop used to
Table 6.1: Average Processing time of packets for Enobio and Truscan with and without artifact removal

<table>
<thead>
<tr>
<th></th>
<th>Processing Time [s]</th>
<th>standard deviation [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enobio</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Stream</td>
<td>0.0375</td>
<td>0.0023</td>
</tr>
<tr>
<td>Artifact Removal</td>
<td>0.1139</td>
<td>0.0214</td>
</tr>
<tr>
<td><strong>Truscan</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Stream</td>
<td>0.0065</td>
<td>0.0017</td>
</tr>
<tr>
<td>Artifact Removal</td>
<td>0.0221</td>
<td>0.013</td>
</tr>
</tbody>
</table>

develop the toolbox, with the following specifications: Intel i5-4200M, 2.50 GHz, 64-bit system with 8 Gb RAM. Performance may vary with different computers based on their specifications as it will affect the processing time of the various different computational loads.

6.6.1 Latency

The latency, here, is defined as the amount of time that is required by the toolbox to compute any packets of data. There are 2 different types of packets, the normal stream is when data is processed without the artifact removal and the artifact removal stream when artifact removal is applied to the data. The processing time of these packets has to be less than the time that the samples of the packets represent.

The Enobio and the Truscan stream data in packets of 200 samples (0.4 s) and 16 samples (0.08 s) respectively. The artifact removal algorithms are applied to the data every 0.4 s. Thus the latency of the normal EEG stream has to be less than the packet sizes for the respective devices and subsequently the latency of artifact removal process has to be less than 0.4 s.

The processing time of each packet was recorded for 5, 2 minute protocols (1 minute of stimulation), for each device. As seen in Table. 6.1, the average latency for both devices and in both modes (normal and artifact removal) is less than the the time of the samples in its packets. Furthermore, for all 2 minute protocols tested here, the latency of all packets was less than the particular thresholds.

6.6.2 Throughput

The throughput, as defined here, is the ability of the toolbox to maintain the latency of the stream over a long period of time. In an ideal system the latency of packets should be maintained indefinitely however due to limitations in RAM and also how Matlab stores data, this is not the case. Matlab allocates space in memory when saving arrays/matrices of data, when more data is added to this array/matrix instead of essentially clipping the data to the end a whole new array/matrix is generated and thus memory is reallocated for this new data. Thereby the processing time for each packet increases with time.
To test the throughput, a 90 minute (5400 s) stream was recorded and the latency of packet computation was recorded for all packets using the Enobio device. As shown in Fig. 6.6, the average latency of the system linearly increases over time and is approaching the crucial limit of 0.4 s when the processing time for artifact removal will be greater than latency. The rate of this linear increase was measured to be approximately 0.004 s per minute and thus would saturate the latency of the Enobio and the Truscan device in 90.62 minutes and 18.38 minutes respectively. This value is much lower for the Truscan device since it streams data in packets of durations of 0.08 s thus the time before the processing time of each packet is greater than the maximum latency is less.

For the use of Enobio, this is not a big issue since no tES stimulation protocols of that length have ever been recorded [2]. For the Truscan, the limit of approximately 18 minutes is not ideal but in the future this can be extended by either increasing the size of the datagram packets by contacting the manufacturer or by exploring options to optimise data storage in Matlab by pre-allocating memory though this will requiring predetermined duration of data to be recorded and will restrict experimental designs to not allow for any unforeseen delays as the data recording will terminate before completion of task in such cases.

6.6.3 Real-time artifact removal performance

The procedures to measure the artifact removal performance using the phantom head model described in Chapter 3 were repeated to measure the performance of the real-time artifact removal using the modified algorithms.

As in the case of offline artifact removal, both the alpha activity induced by eyes closing and the N170 ERPs from the visual task are successfully reconstructed and detected.
Fig. 6.7 and Fig. 6.8 show the examples of a single trial alpha detection and average ERPs for a single simulation with tACS stimulated at 0.5 mA at 5 Hz. However when measuring the correlation coefficients a substantial drop in performance is seen when compared to the offline performance of the methods, see Fig. 6.9.

This is due to the correlation coefficient being very sensitive to the alignment of the signals. Off-line they are perfectly aligned however in real-time operation, although from the same computer is controlling the different hardware, there are unknown and variable delays as the EEG signal propagates through the DAQ. Thus the signals are not 100% synchronised. Correlation coefficients are thus not the best performance metric but are included for direct comparison with phantom head model results from Chapter 4.

Figure 6.7: Real-time artifact removal performance on phantom head - Alpha

Figure 6.8: Real-time artifact removal performance on phantom head - ERP
6.6.4 Summary

The latency and throughput of the toolbox is maintained over short stimulation protocols (less than 20 minutes) for the TruScan and up to 90 minutes for the Enobio. When using the Enobio device, this is not an issue as the longest recorded stimulation sessions using tES are around 45 minutes [2] but for the TruScan device, it is a limitation. However this can be easily fixed by altering the size of the datagrams that the UDP stream sends or by potentially optimising the data storage process within Matlab.

Although a drop in performance of the artifact removal is seen in terms of the correlation coefficients recorded using the phantom head model, the desired evoked responses are still detected using these the real-time variants of the two algorithms and any BCI requiring real-time operation will focus on the detection of said responses. The changes correlation coefficient performances are due to the misalignment of data due to delays in signal propagation and processing.

The real-time artifact removal algorithms can also be further optimised. The SMA algorithm when working in real-time can only account for the past windows rather than the future and past windows and thus any fluctuations in the stimulator output are more likely to result in changes to the reconstructed EEG. For the adaptive filter, in offline function the whole duration of stimulation (1 minute) was filtered at once, however in
real-time operation only packets of 0.4 s are processed in each iteration. Thus in future, parameter of the filter such as filter coefficient and forgetting factor can be adjusted to account for the shorter signal samples or alternatively a trade-off between latency (size per packet) and accuracy may be investigated. These optimisation may improve results. Regardless, as shown by results, in real-time operation both the induced alpha activity and the evoked N170 ERPs can be detected using these methods.

6.7 Summary

The new tACS-EEG toolbox design allows researchers to conduct cognitive experiments with tACS and provide access to real-time data which can be monitored and assessed to adjust stimulation parameters to provide a full closed-loop functionality. This is the first tool that integrates all the different aspects of running such experimental protocols. The toolbox is hardware independent as much as possible, being compatible with any EEG device which makes a TCP/IP or UDP stream available. For the AF the stimulator output is required which is currently only available on a Neuroconn device. Thus the toolbox can be used by researchers at minimal extra equipment costs. This is a significant advantage as the current barrier to entry for conducting real-time EEG+tACS experiments is very high, it requires purchasing expensive equipment (Neuroconn Neuroprax or Neuroelectrics Startsim) which cost approx. £20,000.

The goal is to release this toolbox as an open source package that researchers across the world can access to implement their designed studies. Furthermore, this package will allow researchers to test new algorithms for artifact removal or closed-loop stimulation and compare them to existing techniques. This will allow for an ever improving database of methods that can allow new researchers to test these different methods before selecting techniques best suited to the design of their study.

References


Chapter 7

Conclusions and Future Work

7.1 Contributions

This thesis presents novel methods that allow real-time monitoring of EEG activity during tACS stimulation. New methods for artifact removal were presented and much needed verification techniques were developed to test and validate the performance of these methods. Subsequently the proof of concept for monitoring EEG activity during stimulation was established. This was achieved by presenting, for the first time, working memory monitoring during tACS using EEG.

At the start of my PhD, researchers were starting to identify the need to monitor neural activity during tACS. In general a need to better understand the effects of stimulation was identified and furthermore the need for more targeted stimulation protocols was anticipated. This has only recently been highlighted by the wider research community making the contributions of this thesis particularly timely. This has been recognised by both; the industry, as Rogue Resolution Ltd awarded this study a prize as part of the BrainBox initiative; and in literature, as various studies pointed to the variation in results due to the state dependant efficacy of tACS [1]–[5]. Thus the next generation of stimulation protocols would highly benefit from closed-loop feedback based on ongoing activity.

In chapter 2, a review of existing work and methodologies in the field of tES showed that methods allowing EEG to be monitored during tACS were a key milestone for the development tACS as a tool for both providing insight into the role that neural oscillations play in the brain and also for applications as a therapeutic tool in mental health. Existing design of research studies attempting to utilise EEG data with tACS were investigating effects of stimulation by comparing activity before stimulation and after stimulation only. This has the obvious limitation of not studying the temporal effects of a technique that interferes with ongoing neural oscillations.

Chapter 3 presents novel methods for artifact removal, the SMA and AF. Beamforming filters for tACS artifact removal were introduced by [6] and [7] had applied PCA for artifact removal. Both Neuroconn and Neuroelectrics have also released methods for real-
time tACS artifact removal algorithms, however these are slow to be adopted by com-
nunity since these ‘proprietary’ algorithms are not easily available to verify and there is a
distinct lack of published work presenting findings using these methods. Of the available
methods, only SMA and AF are both suitable and available for use in development of
the closed-loop BCIs. They are channel independent and not restricted to any particular
hardware, though the AF requires the tES stimulator to output the stimulation signal to
provide the noise template. This allows these methods to be integrated in mobile BCIs
that can operate in closed-loop to provide personalised therapy in mental health.

In general the limited number of currently available studies presenting tACS artifact re-
moval do not verify their techniques by comparing to different methods. In the field of
fMRI-EEG artifact removal, studies presenting new methods regularly compare to differ-
ent method and Average Artifact Subtraction (AAS) is used as a baseline of performance
by almost all of these studies. However in tACS artifact removal, no such standard prac-
tice exist and all studies presenting technical methods include behavioural results. As
discussed in Chapter 3, a more quantitative measure is missing.

I have presented new methodologies, in Chapter 4, to overcome this in several ways.
During stimulation the underlying EEG data is unknown, thus it is hard to use the stan-
dard methods of evaluating performance as there is no template to compare the recon-
structed data with. Thus I developed a gelatin based phantom head model that simulated
pre-recorded EEG signals which could be recorded on the surface of the model. Then
tACS stimulation can also be applied on the surface of the phantom head to simulate
realistic corruption of the recorded EEG data and subsequent artifact removal proce-
dures can be verified as the underlying activity is now known. The reconstructed data
can be directly compared with the pre-recorded EEG data that was used to simulate ac-
tivity. Also the quantitative comparison of performance of three different methods us-
ing various different methods of validation and comparison to provide a degree of confi-
dence that the reconstructed data is indeed valid EEG data. This establishes a standard
of rigour that future studies exploring new techniques can use to assess and compare
their results to, the hardware and channel independence of both these methods enables
researchers to easily apply these methods especially once the toolbox is released.

In chapter 5, the ability to assess EEG activity during stimulation was established. This
assessment is a key stage as it allows practical experimental verification of the artifact
removal by successful classification of EEG activity while performing and not perform-
ing tasks during stimulation. I also provide verification of effect of stimulation by show-
ing separation of data while performing the same task with and without stimulation.
Finally it provides a proof of concept that changes in WM task can be detected during
stimulation. The methods were carefully designed to provide ease of translation to non-
pharmacological treatments in the future to provide therapy for working memory perform-
ance enhancement in mental health disorders.
Finally in Chapter 6, the AF and SMA algorithms were adapted to operate in real-time. To implement this and also to initiate a platform capable of performing closed-loop tACS, I built the EEG-tACS Toolbox in Matlab. The toolbox allows users to control stimulation and stream EEG data during tACS and apply real-time artifact removal. It is designed to run various cognitive tasks and also assess the recorded data in real-time. It is capable of adjusting stimulation in real-time to provide a full closed-loop functionality, though testing of this part of the interface remains to be conducted pending certain hardware upgrades. The goal is to allow researchers to use their own hardware and cognitive tasks such that the library of compatible EEG/tACS devices and stimulation protocols is always expanding.

The novel technological methods introduced in this thesis are key steps towards the application of tACS in closed-loop BCIs to provide personalised therapy. Furthermore, the phantom head model is a valuable resource that has applications beyond the course of this study, for example, it can be utilised for characterisation of EEG hardware or artifacts (other than from tES) in controlled environments. Some preliminary work has already been done on this and is included in Appendix C. Also the inclusion of all these techniques in the toolbox provides a platform for other researchers to utilise these methods and build upon them.

### 7.2 Potential future work

#### 7.2.1 Testing the closed-loop interface

The software interface for closed-loop operation using the EEG-tACS toolbox is built. To test it using the available tACS hardware (Neuroconn) an isolated, voltage based, signal generator is required that needs to be integrated to the toolbox such that the adjusted tACS waveforms can be sent to the stimulator. To establish proof of concept, the prepared protocol will aim to:

1. Modify stimulation amplitude based on alpha power measurement during stimulation;
2. Adjust stimulation frequency based on measured individual alpha frequency (IAF) and induce IAF change using the eyes open/close task;
3. Match the phase of individual alpha stimulation to ongoing alpha activity.

#### 7.2.2 Controlled induction of non-linear artifacts

As [8] described, there is now some debate on linear vs non-linear artifacts and the impedance of the tES setup is not constant. Non-linear artifacts are produced due to changes in-
curred by ongoing bodily processes such as heart rate and respiration. Methods like SMA and AF are able to account for these temporal changes as the moving average and the stimulator output captures the changes in the stimulation artifact respectively but there still remains the potential for secondary artifacts to be induced as the temporal resolution for the SMA and AF algorithm may not be enough to entirely suppress these artifacts.

Here, the phantom head model can be utilised to characterise these secondary artifacts and shed further light on this, beyond the current work that employed a melon as the phantom head to characterise these artifacts [9]. The impedance of the phantom head model can be directly manipulated to introduce changes in the stimulator output and thus the secondary artifacts can be introduced and characterised in a controlled environment. Subsequently, techniques can be designed that detect these changes in impedance and thus directly target these induced artifacts. Also, as shown in Appendix. C, the phantom head can also be used to develop other EEG based techniques such as real-time in-band impedance monitoring.

7.2.3 Closed-loop WM enhancement protocols

In Chapter 5, I introduced methodologies that allow for artifact removal during different working memory tasks. Furthermore, post artifact removal, I was able to separate the different working memory states (baseline and two different tasks) post artifact removal using machine learning techniques. Subsequently in Chapter 6, I introduced variations of the SMA and AF algorithms that operate in real-time as part of the EEG-tACS toolbox.

Now methods from Chapters 5 and 6 can be combined for the application of working memory enhancement to explore real-time workload monitoring and algorithms for closed-loop personalised tACS therapy. The next step is to monitor and detect different levels of difficulty during the same task and subsequently adjust the stimulation to account for the higher workloads.

7.3 Summary

I have presented a set of general tools for removing artifacts and characterizing the performance of these methods. Novel methods to monitor working memory during tACS stimulation were presented and an interface that enables doing so in real-time was also designed. These contributions have substantial potential for enabling a number of basic neuroscience, and applied clinical studies in the future.
References


Appendices
Appendix A

Inclusion Questionnaire

Find the inclusion questionnaire used for this study below.
Participant details and inclusion questionnaire

CONFIDENTIAL

Title of project: Study into the use of non-invasive brain stimulation (transcranial alternating current stimulation) in Brain-Computer Interface and working memory applications

Principal investigator: Dr. Alexander Casson

Participant identification number for this study:

    Age:
    Sex:
    Handedness:

Does the participant want to be kept informed of the study results:

1. Do you have epilepsy or have you ever had a convulsion or a seizure?

2. Do you have severe or frequent headaches?

3. Have you ever suffered from brain injury, stroke, or undergone neurosurgery?

4. Have you ever had a head trauma that was diagnosed as a concussion or was associated with loss of consciousness?

5. Do you have cochlear implants?

6. Are you pregnant or is there any chance that you might be?

7. Do you have intracranial (within the head) metals such as clippings, coilings, ventriculo-peritoneal shunts, endoprosthesis etc.?
8. Do you have an implanted neurostimulator (e.g., DBS, epidural/subdural, VNS)?

9. Do you have a cardiac pacemaker or intracardiac lines?

10. Do you have a medication infusion device?

Are you aware of any damaged skin tissue on your head, or do you suffer from a skin condition such as severe eczema, or do you suffer from any skin allergies?

11. Have you ever had any brain related conditions or any mental disorder (e.g. depression, bipolar disorder, schizophrenia)?

12. Do you have any hearing problems or ringing in your ears?

13. Have you ever had a fainting spell or syncope? If yes, please describe on which occasion(s)?

14. Are you taking any medication, or have you taken any medication within the last 24 hours?

15. Have you ever undergone Transcranial Magnetic Stimulation (TMS), transcranial Current Stimulation in the past? This may have been called tDCS, tACS or tRNS.

If so, please state if there were any problems:

---

Participants answering yes to any of these questions may not suitable for this study

Name of the researcher to contact if there are any problems:

Dr. Alexander Casson (alex.casson@manchester.ac.uk)
Appendix B

Ethical Approval

Find ethics approval letter for all experiments used in this study below.
Dear Dr Casson,

Research Ethics Committee 4

[Kohli, Casson: Study into the use of non-invasive brain stimulation (transcranial alternating current stimulation) in Brain-Computer interface and working memory applications. (ref 14263)]

I write to thank you and Mr Kohli for coming to meet the Committee on 23rd July 2014 and to confirm that it gave the above research project, after the submission of amendments / clarifications, a favourable ethical opinion.

This approval is effective for a period of five years and if the project continues beyond that period it must be submitted for review. It is the Committee’s practice to warn investigators that they should not depart from the agreed protocol without seeking the approval of the Committee, as any significant deviation could invalidate the insurance arrangements and constitute research misconduct. We also ask that any information sheet should carry a University logo or other indication of where it came from, and that, in accordance with University policy, any data carrying personal identifiers must be encrypted when not held on a university computer or kept as a hard copy in a location which is accessible only to those involved with the research.

Finally, I would be grateful if you could complete and return the attached form at the end of the project or by the end of June 2015.

We hope the research goes well.

Yours sincerely,

Dr Deborah Bentley
Secretary to University Research Ethics Committee 4
UNIVERSITY OF MANCHESTER

UNIVERSITY RESEARCH ETHICS COMMITTEES

Progress or Completion Report Form on an Approved Project

The Committee’s procedures require those responsible for projects which have been approved by the Committee to report on any of the following:

- Any incident, accident or untoward event associated with the project (Please note that if the incident constitutes an accident or dangerous occurrence, the usual Health and Safety reporting mechanism must still be used)

- Any variation in the methods or procedures in the approved protocol

- A termination or abandonment of the project (with reasons)

- A report on completion of the project or a progress report 12 months after approval has been given.

This form should be completed and returned to research.ethics@manchester.ac.uk

University reference number:

Project title:

Principal investigator:

Date of report:

Progress/completion report (generally this does not need to be longer than half a page):
Appendix C

Continuous in-band electrode impedance monitoring

C.1 Introduction

The electroencephalogram (EEG) is a widely used tool for neuroimaging. It is based upon placing small metal electrodes on the scalp and recording the very small electrical potentials outside of the body due to neuronal action within the brain. It is in principal a small, portable technology and ambulatory EEG units which do not require long recording wires have been available for many years [1]. In the last decade there has been significant interest in wearable EEG devices that are even more miniaturised, easy to use, and allow high quality, real world, neuroimaging for the first time [2]. However, despite this interest and significant progress, there remain many challenges before true real world EEG is realised [3]. For improving data quality and resistance to artifacts the performance of the electrode contact with the scalp is critical. In the ideal case the impedances of all electrodes are equal and this minimises the common mode-to-differential conversion of external interference sources such as 50/60 Hz pick-up and motion. However, historically it has not been possible to measure the impedance of an EEG electrode simultaneously with signal collection. Impedance measurements, and correcting mismatches by adjusting the physical connections, have only been done at the start/end of a recording.

Recently, simultaneous EEG monitoring and impedance measurement has been proposed by recording EEG in the usual manner, and simultaneously injecting a small current at \( \sim 1 \text{ kHz} \), out of the EEG frequency range of interest[4], Figure. C.1. The out-of-band signal can be isolated in the instrumentation electronics and used to calculate the contact impedance at 1 kHz while leaving the lower frequency EEG trace unaffected. However, this relies on having a good model of the electrode–scalp contact to allow the impedance at physiological frequencies (5–30 Hz) to be inferred. This is challenging, because the actual electrode contact model varies over time with the amount of hair present, the scalp condition, the amount of sweat and similar. In this paper we investigate techniques to allow the contact impedance at 5–30 Hz to be measured directly while leaving
minimal residual artifacts in the collected EEG trace. In the methods section we present a new notch filter for removing in-band interference, and a signal processing enhanced method using a combination of hardware and software to remove the artifact. The performance is assessed in Section C.3 and the second method in particular allows a continuous EEG impedance measurement, giving greater real-time insights into the quality of the EEG, and is the first step towards a dynamic EEG front-end which could alter its electrode impedance in real-time to automatically balance the impedance between electrodes.

C.2 Artifact removal methods

The basis for simultaneous EEG and contact impedance measurement is shown in Figure. C.1. In conjunction with a standard EEG amplifier a current source is connected to the electrode and drives a small current into the body. A typical waveform used for the impedance signal at 15 Hz is shown in Figure. C.2a. This signal mixes with the true EEG and when both are recorded at the same time leads to an artifact corrupted EEG trace, Figure. C.2b. As the frequency of the impedance signal is known it can be isolated and the recorded amplitude and phase at this frequency used to calculate the impedance. Conventionally the EEG collected at the same time is not used as it contains artifacts from the impedance measurement. Such artifacts can be avoided by performing the impedance measurements at out-of-band frequencies, typically 1 kHz. Here we consider two methods for removing impedance measurement artifacts applied in-band at frequencies from 5 to 30 Hz.

C.2.1 Notch filter method

The first approach is based upon a new ultra low power high order notch filter for removing the specific in-band frequency that the stimulation artifact is present at. For low power, low voltage, low frequency operation a \( g_mC \) filter structure is used as its power consumption is directly proportional to the cut-off frequency, which is itself very low.
Figure C.2: (a) 15 Hz signal used for measuring contact impedance. In practice the superposition of two components either side of 15 Hz are used to give improved robustness. (b) Simultaneously collected EEG (recorded using the head model in the performance section shows artifacts due to the impedance measurement.

Figure C.3: Fourth order $g_{m}C$ notch filter circuit based on a doubly terminated LC ladder. All transconductors are the same (Figure. C.4).

(5–30 Hz for in-band EEG). A fully differential fourth order topology is shown in Figure. C.3 with $Q = 0.735$. The design is based on a doubly terminated LC ladder prototype as this structure gives the minimum sensitivity to process and device mismatch when operating in weak inversion [5].

Capacitors are sized for a transconductance in the range 1–10 nS, and the used transconductor is shown in Figure. C.4. For low power, low transconductance operation it has a folded cascode structure with input cross-coupling for transconductance reduction [6]. A nominal bias current of 512 pA is used, which can be tuned to provide the wanted impedance rejection frequency. This means that transistors are biased in the deep weak inversion region [7], minimising power at the cost of bandwidth. The low frequency nature of EEG (<100 Hz) means that the required bandwidth is readily achieved via suitable transistor sizing. The whole circuit operates from a 1.3 V supply so can be directly driven from a single coin cell battery. Implemented in a triple well, 0.18 µm CMOS process with MIM capacitors the layout is shown in Figure. C.5 and extracted simulation performance in Table C.1.
C.2.2 Superposition of Moving Averages method

The second approach is based upon digital processing. Superposition of Moving Averages (SMA) was originally developed for removing artifacts of transcranial a.c. current stimulation (tACS) from simultaneous EEG measurements [8]. In tACS rubber scalp electrodes non-invasively inject currents into the head at cortical frequencies, typically between 5 and 40 Hz, in order to directly modulate the on-going neural activity. Typically currents up to 2 mA_{pp} are used, and these produce a very large artifact signal which obscures the EEG trace. In principle in-band impedance measurements are exactly the same, but inject much smaller sub-\(\mu\)A currents which do not modulate the ongoing operation of the brain, and give a similar but smaller artifact to reject.

The SMA formulation used here is given in Figure. C.6 [8]. The length of the impedance artifact is known and so the EEG trace is segmented into \(N\) epochs of this duration, and \(M\) such epochs are averaged together such that the EEG signal content averages to zero while the amplitude of the periodic impedance artifact is maintained. This allows the generation of a channel and time specific template of the impedance artifact, which

<table>
<thead>
<tr>
<th>Table C.1: Performance of the 4th order notch filter.</th>
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<tbody>
<tr>
<td>CMOS process</td>
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<tr>
<td>Supply voltage</td>
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<tr>
<td>Typical bias current</td>
</tr>
<tr>
<td>Area</td>
</tr>
<tr>
<td>Power consumption</td>
</tr>
<tr>
<td>Signal input range</td>
</tr>
<tr>
<td>THD (100 mV_{pp}, 10 Hz)</td>
</tr>
<tr>
<td>Dynamic range</td>
</tr>
<tr>
<td>Tuning range</td>
</tr>
</tbody>
</table>
Figure C.6: Algorithm for SMA. Data from each channel is separately segmented before a moving average artifact template is calculated, which is then subtracted from the original data recorded for that channel.

Figure C.7: Proposed signal processing assisted amplifier for in-band impedance measurements.

evolved with time and intrinsically scales with the number of EEG channels used. The scheme is formulated so that the artifact removed EEG is generated by subtracting the current artifact template from the current EEG. In this work this is done in software in the digital domain. It is anticipated that in the future this can be implemented as in-the-loop signal processing, suppressing the artifact as it is collected, Figure C.7. Here $M$ is selected to be 5% of $N$ (the total number of impedance measurements) as a suitable trade-off between the number of averages taken and the time localization of the artifact template.

C.3 Performance comparison

To assess performance an EEG head model phantom, similar to that in [9], was used to simulate simultaneous EEG and in-band impedance measurements. Electrodes were implanted inside head shaped conductive gelatine and pre-recorded EEG sized signals played into the model from a data acquisition unit. This allowed known EEG signals to be present at the surface of the head and provide a known comparison signal for assessing the EEG data quality during impedance measurement. The experiment set up is shown in Figure C.8 where EEG electrodes are held in place using EC2 adhesive (Natus Medical, USA). Two electrodes on the mid-line (FCz, Cz) were used as EEG ground and reference and connected to an Enobio (Starlabs, Spain) ambulatory EEG unit, with a recording channel on the rear of the head in location P4. These were simultaneously...
Figure C.8: Head phantom used to provide a known reference EEG during simultaneous EEG and in-band impedance measurements.

connected to a SIGGI (EasyCap, Germany) stand alone impedance meter which injected an impedance current at the desired frequency and allowed the impedances to be measured during the EEG acquisition. The collected signals were then processed using the notch filter approach or the SMA approach to remove the artifact of the impedance measurement.

In all test set ups the impedance was measured continuously, with impedance values being constant at approximately 1.2 kΩ. An illustrative example of the impedance artifact removal process is shown in Figure. C.9 for an in-band impedance measurement at 5 Hz which highly overlaps with cortical frequencies of interest in the 5–30 Hz range. Figure C.9a shows a section of EEG data recorded from the head model, which includes artifacts from the impedance measurement process (as in Figure C.2). Figure C.9b shows the known EEG trace which would be recorded in the artifact free case, and Figure C.9c and d show the processed EEG data after SMA and notch filter methods respectively. Both Figure. C.9c and d follow the general trend of the EEG signal shown in Figure. C.9b, for example with a large negative component at time 47 s. Within this, the SMA method closely follows the desired EEG trace, with more residual artifacts present after notch filtering, which is expected as it also removes cortical frequencies of interest.

The performance is quantified in Table C.2 for in-band impedance measurements at 5, 15 and 30 Hz. Table C.2 measures the correlation coefficient between the known EEG trace and the artifact processed EEG traces over 2 minute recordings which include pre-recorded alpha activity, that is, bursts of EEG in the 8–12 Hz range. This input is deliberately chosen to be directly adjacent to the impedance measurement frequencies giving a challenging signal recording and artifact rejection case. Using SMA, for 5 Hz and 15 Hz impedance measurements the correlations are very high, >0.9, showing that the EEG is correctly recorded despite the presence of an in-band impedance artifact in the raw trace. The performance of the notch filter is worse, particularly for the low 5 Hz impedance measurement. To put these figures in context, typical correlation coefficients when comparing the performance of different EEG electrode types are: >0.93 [10]; 0.89 [11]; 0.83 [12]; 0.81–0.98 [13]; 0.68–0.90 [14]; 0.39–0.85 [15]. Using both in-band
Figure C.9: Example removal of impedance artifact for a 5 Hz in-band impedance measure.

Table C.2: Correlation coefficients between the known EEG trace and the artifact processed traces.

<table>
<thead>
<tr>
<th>Method</th>
<th>Impedance measurement frequency / Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>SMA</td>
<td>0.94</td>
</tr>
<tr>
<td>Notch</td>
<td>0.79</td>
</tr>
</tbody>
</table>

impedance methods the correlation coefficients are larger than these, indicating a minimal loss of information, equivalent to changing the electrode type used. The SMA approach consistently outperforms the notch filtering method with high correlation coefficients obtained at all impedance measurement frequencies.

C.4 Conclusion

Electrode contact impedance measurements are an important assessment of data quality in EEG recordings, but have not previously been possible to obtain simultaneously with EEG at the wanted frequencies in the 5–30 Hz range. This paper has demonstrated in-band simultaneous impedance monitoring for the first time and showed that minimal residual artifacts are introduced in the EEG trace, with correlations compared to a gold standard reference of up to 0.94.
Bibliography


