The entrainment of brain oscillations through transcranial alternating current stimulation (tACS) delivered at off-peak frequencies.

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The usage of the word ‘peak’

Throughout this thesis the term ‘peak’ is used to refer to the dominant frequency within a given frequency band. Whilst, outside of the alpha band, the dominant frequency rarely forms what would be considered a ‘peak’ in the traditional sense, this term has been used for all frequency bands throughout this thesis for simplicity. This term has also been used interchangeably with the phrase ‘average frequency’.

Abstract

It has been demonstrated that endogenous brain oscillations can become entrained to rhythmic stimulation. Four studies were conducted to investigate the possibility of modulating endogenous frequency peaks, through entrainment, from stimulation delivered at off-peak frequencies. Study 1 utilised EEG recordings to establish whether off-peak stimulation within the alpha frequency range could successfully shift individual alpha peak frequencies in the direction of stimulation. Stimulation was delivered at 2Hz above and below the individual alpha peak frequency of each participant. EEG recordings taken immediately after stimulation found no significant modulation of individual alpha peak frequencies. In light of this, the subsequent studies assessed the effects of off-peak stimulation during the stimulation rather than immediately following it. These studies used a working memory task and were based on the theory that differences in theta and gamma frequency peaks causally effect working memory capacity. Studies 2 and 3 delivered tACS at off-peak theta frequencies (4Hz and 7Hz) and study 4 delivered tACS at off-peak gamma frequencies (40Hz and 70Hz) during a working memory task. This was done to ascertain whether such stimulation could alter working memory capacity, which would indicate that peak frequencies had been shifted. Studies 2 and 3 utilised two different electrode configurations (P4/Cz and P4/right supraorbital respectively). In study 3, working memory capacity was enhanced

and impaired in the 4Hz and 7Hz conditions respectively. In studies 2 and 4, no significant changes in working memory capacity were found. The findings from study 3 indicate that off-peak entrainment can be used to shift frequency peaks in the direction of the stimulation frequency and that such shifts can have observable cognitive effects. The contrast between studies 2 and 3 highlights the importance of electrode placement in tACS studies.

Chapter 1: Brain oscillations

1.1 Mechanisms of brain oscillations and the principles of EEG recordings

The difference in the electrical charge of the fluid inside neurons compared to that of the fluid outside of neurons (a result of the different concentrations of ions and anions) is known as the membrane potential (as it occurs across the cell membrane) (Hammond, 2015). The fluctuations of a membrane potential at the post-synaptic neuron are referred to as the post-synaptic potential. The post-synaptic potentials of one neuron can be recorded from depth electrodes and such recordings have been performed on animals and on humans during neurosurgery yet, due to the invasiveness of the procedure for single neuron recordings, this technique has ethical limitations. However, when a large number of neurons are oscillating in synchrony with each other, known as phase-coupling, the summation of post-synaptic membrane potentials produces a signal strong enough to be recorded non-invasively through electrodes placed on the surface of the scalp (Jackson, 2014; Schaul, 1998). The recording of these summations of post-synaptic potentials through electrodes on the scalp is known as electroencephalography (EEG) (e.g. Schaul, 1998).

The first documented EEG recording was made by Richard Caton on exposed monkey and rabbit brains in 1875 (Collura, 1993). A further report by Caton in 1877 noted that differences were observable between different states of awareness such as wakefulness, sleep and anaesthesia. Approximately 50 years later Hans Berger recorded the first human EEGs. The first published report of a human EEG was by Berger in 1929, 5 years after Berger first began conducting human EEG recordings (Collura, 1993).

Whilst EEG recordings are the results of summations of membrane potentials from groups of synchronised neurons, at any given time there are multiple groups of phase-coupled neurons each producing a recordable signal. EEG data is a collection of these signals (Cohen, 2017), meaning that in most instances a large portion of EEG data will be superfluous to the requirements of the recording. There are two main methods of analysing EEG data; event-related techniques and frequency analyses. Both of these involve averaging certain aspects of the raw data, thus allowing the aspect of interest to be focused on by cancelling out the 'noise' of superfluous signals. (See figure 1 for an example of typical EEG data prior to any averaging.)

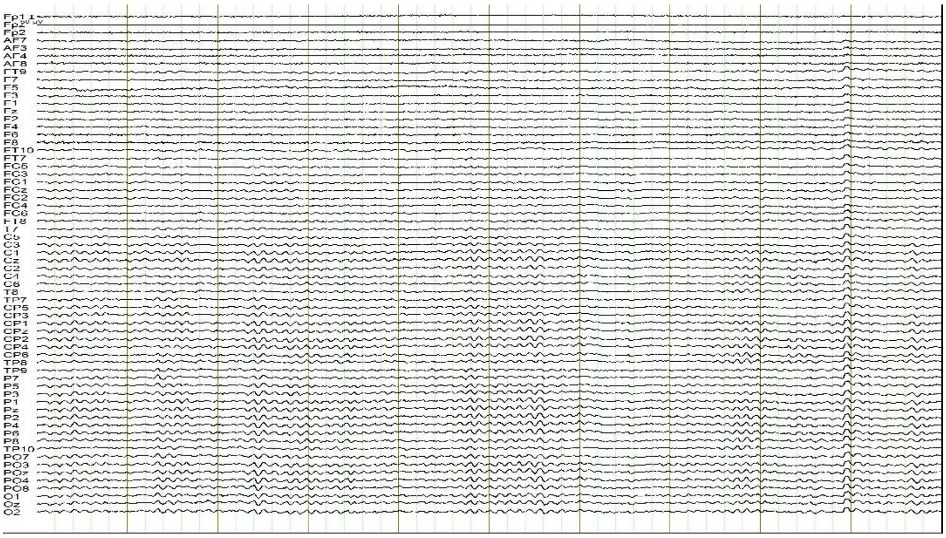

Figure 1. Raw EEG data

1.2 Types of EEG analysis

The most common form of event-related analysis is calculating event-related potentials (ERPs) although event-related synchronisations (ERS) and event-related desynchronisations (ERD) are becoming more commonplace (these latter two will be outlined below). ERP studies involve recording EEG data whilst a particular 'event' (such as a stimulus or a participant response) is repeated. The raw EEG data is then separated into a segment (epoch) for each occurrence of the event, with each segment time-matched to its respective event. These epochs are then averaged to remove random noise (through cancellation). This provides a waveform which is the averaged EEG data occurring during the given event; this is the ERP (Schneider et al., 2018) (see figure 2a). As the task irrelevant noise has been cancelled through averaging across epochs, the ERP provides an indication of the EEG correlates that are relevant to the particular event being studied. In most ERP studies control conditions allow for the cancellation of task relevant but condition irrelevant EEG correlates (such as low-level vision related ERPs that may occur in response to a visually presented stimuli) (Kappenman and Luck, 2015). This provides clearer, less contaminated data, however, it does result in large quantities of data being discarded. One issue with this type of data discarding is that all variance is treated as 'noise' and it is not possible to distinguish between genuine random noise and variations that may be more relevant to the research being conducted (Kappenman and Luck, 2015). A more practical issue related to this problem is that large amounts of data need to be collected[1] in order for a relatively small amount of useable data to be produced (which can also lead to the additional problem of an increased likelihood of finding falsely significant effects) (Luck & Gaspelin, 2017).

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[1] This is usually in the form of a repetitive task being carried out by the participant, something that in itself can produce further problems such as fatigue, disengagement and practice effects.

Frequency based analyses average raw EEG data using a power analysis across frequency points. The most commonly used technique is the fast Fourier transform (FFT) (Al-Fahoum & Al-Fraihat, 2014). This method converts the raw EEG data into a frequency x power graph (see figure 2b.) As with ERPs, the raw data is separated into epochs of a particular duration. However, in this case they are not time linked to an event. The power of each particular frequency point (often calculated in bins of 0.125 or 0.25Hz depending on the original sampling frequency and size of the epoch) is then calculated for these epochs and then averaged to produce a graph showing the average power of each frequency point. When multiple frequency plots are compared (such as in two different conditions) it gives an indication of the relative synchronisation (power increase) or desynchronisation (power decrease) occurring within different frequency bands between conditions.

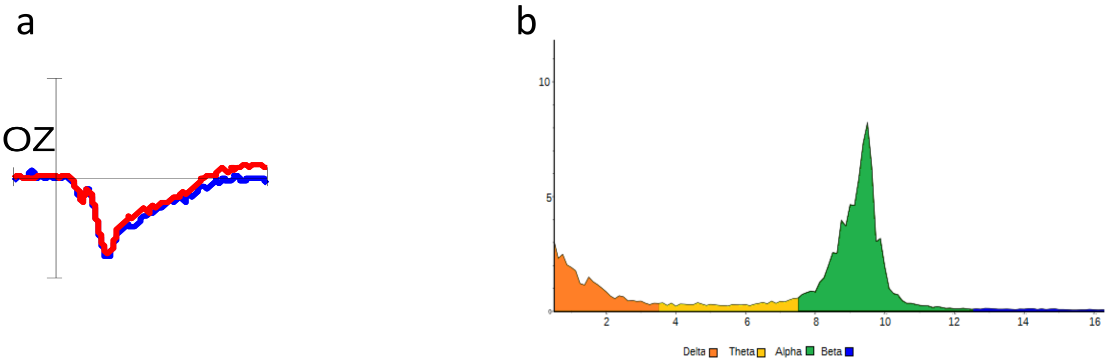

Figure 2. a) Two raw ERP waveform plots overlaid for comparison. b) A frequency x power plot produced following an FFT on EEG data

ERS and ERD combine the time-locked aspect of ERP analysis with frequency analysis to produce an indication of mass synchronisation or desynchronisations that may occur in response to an 'event'. The analysis is similar to that of the basic frequency based analysis outlined above, however the epochs used are time-linked to an event as they are with an ERP analysis. Whilst the technique (and therefore the data) differs from the technique of an ERP analysis, there is some debate regarding whether ERPs and ERS/D are neurophysiologically distinct from each other or are merely different ways of looking at the same thing. ERPs are believed to show a mass phase-resetting of neural oscillations in response to an event and ERD/ERS is believed to show a mass phase-locking in response to an event. There is currently a debate about whether, neurophysiologically speaking, phase-locking and phase-resetting are the same thing (e.g. see Klimesch, Sauseng, Hanslmayr, Gruber & Freunberger, 2007; Schneider & Maguire, 2018).

1.3 Frequency bands in EEG

Traditionally, the different oscillatory signals from EEG recordings have been divided into broad categories based on their frequencies. These categories are known as frequency bands and have various functional correlates associated with them. However, these frequency bands can at times be over-simplistic or not match neatly with a particular function. For example, a particular function may span the cusp of two separate frequency bands (e.g. Waldhauser et al., 2012) or may only occur in a smaller sub-range of a particular frequency band (e.g. Klimesch et al. 1998). Furthermore, different distinct brain rhythms have been identified within the same category band.

For example, the sensorimotor mu rhythm and the posterior alpha rhythm both fall under the alpha frequency band (8-12Hz) yet are two very distinct rhythms, originating from different cortical regions. Under the traditional frequency band system these are both considered alpha activity, yet this misleadingly combines these two distinct neural rhythms. This highlights that other factors, such as the topography (Kropotov, 2009) and waveform shape (Cole & Voytek, 2007; Kropotov, 2009) of rhythms need to be taken in to account when categorising them.

Despite this, these frequency band categories are a useful categorisation system that is appropriate in many cases. The main traditional frequency bands are delta (<4Hz), theta (4Hz-7Hz), alpha (8Hz-12Hz), beta (12Hz-30Hz) and gamma (>30Hz) although there are slight variations in the parameters across the literature. For example, Moran et al., (2010) use a theta range of 4Hz-8HZ, in Tuladhar et al., (2007) the alpha range is given as 8Hz-13Hz, in Tinkhauser et al., (2018) a beta range of 13Hz-35Hz is used and in Axmacher et al., (2010) the gamma range is given as 25Hz-100Hz. In some cases when investigating a smaller subset of a frequency band, the parameters may be extended to reflect the focus of the investigation (e.g. in Klimesch, 1998, the lower alpha range used is 6.5Hz – 10.5Hz and the upper alpha range used is 10.5Hz - 12.5Hz). These frequency bands are sometimes subcategorised in relation to a more specific frequency (e.g. high-gamma; Holz et al., 2010) or in relation to a specific location (e.g. Morgan et al., 2013; Tuladhar et al., 2007) or in terms of a specific waveform shape that occurs at a given frequency (e.g. the sensorimotor mu rhythm; a subset in the alpha frequency range that occurs across the motor cortex (e.g. Cooper et al., 2012) and has a waveform shape that resembles the Greek letter mu (Kropotov, 2009).

Whilst these frequency bands provide a useful categorising system, classifying neurophysiological markers of activity by frequency alone can sometimes be misleading. It has been suggested that these parameters can be too rigid in some cases. Many studies have found functional differences between subdivisions within bands. For example, Klimesch, et al., (1998) found functional differences between low and high alpha activity (6.5Hz -10.5Hz and 10.5Hz – 12.5Hz respectively) and studies have used high and low gamma distinctions when investigating working memory performance (e.g. Alekseichuk et al., 2016; Holz et al., 2010; Sauseng et al., 2008, 2009). Additionally, there have been instances when activity commonly associated with a particular band may fall outside the predefined parameters. For example, in the average healthy adult, a peak in power occurs at roughly 10Hz (i.e. in the middle of the alpha band), however, Haegens et al. (2014) demonstrated that this peak can shift to a higher frequency when the person is engaged in a task typically associated with activity in the alpha frequency (e.g. during an attentional inhibition task). As a result of their findings, Haegens et al. suggested that the commonly used 8Hz-12Hz alpha range may impair the detection of alpha peaks in certain instances where the alpha peak falls outside this range.

Furthermore, the commonly used frequency band categorisations are based on typical adult brain activity and do not always map onto neurophysiological activity from children, the elderly and people with certain neurological conditions. For example, in elderly participants the alpha peak is typically lower in frequency (Scally et al., 2018) and in children, activity that can functionally be considered alpha activity falls within the frequency range that would be considered theta in the neurotypical adult brain

(Chiang et al, 2011). Some examples from the neuropathology literature include alpha frequency peaks being found to be lower in people with autism (Dickinson et al., 2018) and in people with a particular gene expression linked to an increased risk of developing Alzheimer's disease (de Frutos-Lucas et al., 2018). In the same study by de Frutos-Lucas and colleagues, a positive correlation was found between high levels of physical activity and higher alpha frequency peaks (although no causality in this relationship could be determined from this study) indicating further reasons for individual differences in frequencies that may go unchecked even after factors such as age and pathologies are accounted for. Although some of these studies focused on alpha peaks (as opposed to alpha ranges), they all highlight individual differences in frequency ranges that can occur across different populations and their findings indicate the need for flexibility when defining frequency bands in order to avoid missing oscillatory activity that falls outside the predetermined parameters for the frequency range of interest.

To circumnavigate these issues, it has been suggested that tailoring the definition of frequency bands to the individual frequency peaks of each participant would be a more appropriate alternative to the rigidly defined frequency categories currently in use (e.g. Haegens, et al., 2014; Klimesch, 1999; Klimesch, et al., 1998).

A brief overview of some of the main functional correlates of the different frequency bands will now follow:

1.3.1 Alpha (8-12Hz)

The alpha rhythm is so called because it was the first rhythm to be identified, first being noted by Hans Berger in his early EEG studies (Collura, 1993) as a distinct rhythm between 8Hz-12Hz (Buzsaki & Draguhn, 2004). Although the alpha frequency band is commonly considered to range from 8-12Hz, as mentioned above, the frequency of alpha activity changes throughout the lifespan, being lower in frequency in both childhood and old age (Chiang et al, 2011; Scally et al., 2018). In addition to a decrease in frequency, alpha activity also decreases in power in old age (e.g. Babiloni et al. 2006).

Historically, alpha has been thought to reflect cortical idling and a resting brain state as alpha synchronisation increases when a cortical area is at rest and desynchronisation occurs when engaged in a task (e.g. see Pfurtscheller et al., 1996). Furthermore, the extent of desynchronisation has been linked to task difficulty, e.g. Van Winsum et al., (1984), who showed that increased task difficulty led to an increase in alpha desynchronisation. Additionally, alpha activity is particularly dominant in occipital regions and this occipital alpha increases in amplitude when the eyes are closed, further supporting the idling theory of alpha (Kropotov, 2009). However, a more complex account of alpha activity has since emerged with it now being thought to play a more active role, particularly in areas involving inhibition (see Cooper et al, 2003 for a discussion of this, also see Klimesch, Sauseng & Hanslmayr, 2007).

It has been shown that an increase in alpha power occurs during tasks that require heavy inhibition of irrelevant information. For example, in a series of experiments, Cooper et al., (2003) found an increase in alpha power when greater inhibition was required due to increased task demands, and in a study by Sauseng et al. (2009) it was found that greater alpha power was predictive of a greater ability to inhibit irrelevant material during a short-term memory task. Furthermore, in the same paper, Sauseng and colleagues demonstrated a causal link between alpha power and inhibition. In the study, Sauseng and colleagues increased alpha power through rhythmic transcranial magnetic stimulation (rTMS) (delivered at the alpha frequency of 10Hz), which subsequently led to an increase in the successful suppression of irrelevant material during a short-term memory task. Alpha tACS has also been shown to decrease the impairments of cognitive inhibition that are associated with aging (Borghini et al., 2018), providing further causal evidence for the link between alpha activity and inhibition and the link between declining alpha power and age.

The mu rhythm is an alpha rhythm centred around 10Hz which occurs over the sensorimotor strip and has a waveform shaped like the Greek letter mu (Kopotov, 2009). It has been shown to increase in power when muscles are relaxed or idling (e.g. Pfurtscheller, et al., 1996) and to decrease in power during movement (e.g. Hobson & Bishop, 2016), observation of movement (e.g. Cochin et al., 1999) or when tactile stimulation occurs (e.g. Gaetz & Cheyne, 2006) linking it to both tactile sensations and motor activity, as well as the mirror neuron system.

In addition to being linked to inhibition, alpha oscillations have been implicated in multisensory processing. Cecere et al., (2015) found that alpha cycles play a role in binding together items from different senses so that they are able to be interpreted as one cohesive stimulus. If the information received from both senses falls within one alpha cycle the two stimuli are perceived as linked, whereas if they fall across two different alpha cycles they are perceived as separate from each other.

1.3.2 Beta (12-30Hz)

Unlike alpha, the majority of people do not exhibit a clear peak within the beta frequency range (Kropotov, 2009). Beta activity is found in multiple areas across the brain, however, historically, the beta activity across the sensorimotor area is the most well researched (Spitzer & Haegens, 2017). As to be expected given its position, the sensorimotor beta rhythm is associated with movement. Specifically, it has been linked to voluntary movement and its initiation and suppression. Desynchronisation of sensorimotor beta activity is found to occur before and during voluntary movement (e.g. Gilbertson et al., 2005; Kuhn et al. 2004; Pfurtscheller, 1981; Pfurtscheller et al., 1996) with a rebound synchronisation occurring afterwards (Pfurtscheller et al., 1996; Pfurtscheller et al., 1998) and synchronisation of beta activity correlates to a decrease in the initiation of movement (Gilbertson et al., 2005). An increase in beta synchronisation has been linked to Parkinson's disease (Eusebio and Brown, 2009), a condition where the initiation of voluntary movement is impaired and has also been implicated in tic suppression with people with Tourette's syndrome (Niccolai et al. 2016) i.e. beta synchronisation occurs during the suppression of 'semi-voluntary' movements[2]. The sensorimotor beta rhythm often co-occurs with the alpha mu rhythm,

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[2] Tics in Tourette's syndrome and other tic disorders are referred to as semi-voluntary. This is because tics are volitional rather than automatic reflexes, yet performing a tic eases discomfort and not carrying out a tic increases discomfort. Supressing a tic can be likened to somewhere between supressing a sneeze and not scratching an itch.

yet comparisons have shown that sensorimotor beta and mu are generated from different sources and during the rebound synchronisation of sensorimotor beta after voluntary movement, the mu rhythm remains desynchronised (Pfurtscheller et al., 1996).

Beyond the sensorimotor beta rhythm, the role of beta is less clear, yet beta activity is found in multiple locations beyond the sensorimotor area and this activity has been linked to a wide range of cognitive functions (see Spitzer and Haegens, 2017 for a review). For instance, beta activity has been associated with perceptual decision making (Alavash et al., 2017), maintenance of the current cognitive state (Engel & Fries, 2010), working memory (Deiber et al., 2007) and language processing (Weiss & Mueller, 2012) amongst other functions.

In their review, Spitzer and Haegens (2017) propose that beta activity is involved in endogenous processing of task relevant information, a theory which could bring together some of the seemingly disparate functions of beta oscillations.

1.3.3 Theta (4Hz – 7Hz)

Oscillations in the theta frequency range are the dominant rhythms in children, with these oscillations moving towards the alpha frequency range by early adulthood. However, it is contested as to whether this activity in children should be considered ‘theta’ as the frequency band ranges were created based on a neurotypical adult brain and these ‘theta’ oscillations more closely resemble alpha oscillations in adults (e.g. Chiang et al, 2011; see also above).

Subcortically, theta activity is present in the hippocampus and limbic system (e.g. Buzsaki, 2002) and two well-researched cortical pathways of theta activity are frontal midline theta (e.g. Zakrzewska & Brzezicka, 2014) and frontoparietal theta (e.g. Polania, 2012). All three of these theta rhythms have been linked to memory processes (Buzsaki, 2002; Klimesch, 1999; Polania, 2012; Zakrzewska & Brzezicka, 2014).

Studies have shown a link between theta activity and memory performance, with greater theta power correlating to increased memory load (Zakrzewska & Brzezicka, 2014) and higher rates of encoding (Klimesch et al., 1996), theta stimulation increasing memory performance (e.g. Polania et al., 2012) and individual differences in theta corresponding to individual differences in working memory capacity (Zakrzewska & Brzezicka, 2014) (also, see Klimesch, 1999, for a review of some of the literature regarding theta activity and memory). Theta activity also plays a large part in Lisman and Idiart's oscillatory theory of short-term memory capacity: the theta-gamma phase coupling theory (Lisman & Idiart, 1995). This theory suggests that theta oscillations work as a binding mechanism, allowing items to be continuously stored in working memory over the course of multiple oscillations (this theory forms the basis for studies 2-4 and will be discussed in more in depth in chapter 5, including a detailed account of the theory's implications for the role of theta activity in short-term/working memory).

In addition to being linked to memory, theta activity is associated with focused yet relaxed states including (but not limited to) hypnosis and meditation. For example, theta activity has been found to increase during Zen meditation (Takahashi et al., 2005) and during hypnosis in participants with high susceptibility to hypnosis (Jamieson & Burgess, 2014) and participants with both high and low susceptibility to hypnosis (Sabourin et al., 1990). Highly hypnotisable people have also been shown to display higher levels of theta synchronisation both before and during a hypnotic state compared to less hypnotisable people (Sabourin et al., 1990).

Theta levels have also been linked to trait anxiety levels. In one study, personality tests were taken by all participants and then frontal midline theta activity was measured during an arithmetic task. Those showing the highest theta levels during the task were found to exhibit the lowest levels of anxiety and neuroticism and the highest extraversion levels, with the inverse being the case for participants showing the lowest levels of theta activity (see Inanaga, 1998).

1.3.4 Gamma (>30Hz)

Gamma activity had been suggested as a mechanism for object representation; bringing together different aspects of an object which are encoded across various spatially separated groups of neurons and binding them into a representation of a whole object (e.g. Tallon-Baudry & Bertrand, 1999). This is known as the binding problem. As well as the binding of different aspects of a whole object, the binding problem can also refer to the question of how perceptions and thoughts can combine to produce conscious awareness (e.g. Revonsuo,1999; Singer, 2001), this is not surprising given that linking various aspects of a stimuli into a cohesive unit can be viewed as an important role in the creation of a conscious awareness of our surroundings (Engel et al., 1999). Thus, gamma activity has been proposed as a potential neural correlate of consciousness (e.g. see Bob, 2011, chapter 2; Buzsaki, 2006, chapter 9; Engel et al., 1999 and Llinas, et al., 1998, for overviews on the study of gamma activity and consciousness). The role of gamma activity in consciousness is also supported by studies such as Mikulan and Hesse et al. (2018) who found greater connectivity in the high gamma range (90Hz – 120Hz) during waking (i.e. a state with conscious awareness) compared to deep sleep (i.e. a state with no conscious awareness). Llinas and Ribary (1993) also found more gamma activity to be present during REM sleep (as well as during waking) compared to deep sleep further indicating that the presence or absence of conscious awareness may be linked to these differences in gamma activity. In this study, Llinas and Ribary also found that the gamma activity during waking responded to external stimuli whereas the gamma activity during REM sleep did not react to external stimuli, mirroring the way in which conscious awareness reacts to external stimuli during waking states but not during REM sleep. A reduction of gamma activity has also been found to be correlated with a loss of consciousness during seizures (e.g. Leeman-Markowski et al., 2015). Bola et al. (2018) found higher rates of localised gamma coupling in deep sleep compared to REM sleep and also in Macaques under propofol anaesthesia compared to macaques under ketamine anaesthesia[3], leading them to propose high rates of local gamma coupling as a mechanism for reduced consciousness (the authors proposed that this hyper-coupling of gamma activity may disrupt localised processing).

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[3] Anaesthesia with propofol appears to result in no conscious awareness, whereas anaesthesia with ketamine results in a dream-like state of awareness, therefore this comparison has similarities to the comparison between deep sleep and REM states.

Gamma activity has also been implicated in memory processes. Gamma activity has been shown to be enhanced when previously encoded items are perceived, this is the case when such items are stored in both STM (Lenz et al., 2008) and LTM (Herrmann et al., 2004). Gamma oscillations also play a major role in Lisman and Idiart's theta-gamma theory of short-term memory (Lisman & Idiart, 1995), with individual gamma cycles being proposed as encoding for individual items in short-term memory. (This theory forms the basis for studies 2-4, with study 4 being particularly relevant to gamma oscillations, and will be discussed in more detail in chapter 5).

1.3.5 Delta (<4HZ)

During wakefulness in healthy human adults delta activity is not visible in the raw EEG trace. In humans, delta waves are the dominant wave type during deep sleep and certain pathological states including coma (Amzica & Kroeger, 2011). They are also the dominant type of oscillation in lizard brains and have been linked to the dopaminergic reward system in humans (See Knyazev, 2007 for a review on this). Delta activity has therefore been associated with lower level brain processing.

Applying an FFT to raw EEG data allows for a more nuanced exploration of specific frequencies, including those that are not visible in the raw EEG trace. In the case of delta, research utilising FFTs have demonstrated an increase in delta power during internal mental processing, such as mental calculation and certain aspects of WM processing and it is thought that this increase in delta power may reflect an inhibition of the processing of sensory information that may otherwise interfere with the internal processing task being performed (Harmony, 2013).

1.4 Individual differences in average frequencies

Within a frequency band, an average frequency can be calculated by multiplying the power by the frequency at regular frequency intervals to create weighted values for these frequency points, and then calculating the average of these weighted values. Individual differences exist in these average frequencies. For example, the average alpha frequency is generally given as ~10HZ (e.g. Klimesch, 2012), however this exact value varies across individuals (Klimesch, 1999).

Individual differences in average frequencies have been shown to be correlated to individual differences in cognitive functions that are related to the given frequency band. For example, differences in individual alpha peaks (Individual alpha frequencies – IAFs) have been linked to individual differences in various aspects linked to intelligence, such as verbal abilities (Anokhin & Vogel, 1996) and memory (e.g. Clark et al, 2004; Klimesch, et al., 1990; Klimesch et al., 1993). Some studies have found a link between IAF and overall general intelligence (*g)* (e.g. Grandy et al., 2013), however, this correlation is contested, with other studies finding no evidence that differences in IAF relate directly to differences in *g* (e.g. Anokhin & Vogel, 1996). Furthermore, findings from studies such as Axmacher et al. (2010), who found that differences in working memory load have been linked to modulations in theta peaks, along with the theoretical and empirical support of the role of theta-gamma phase coupling in STM/WM capacity

(Lisman & Idiart, 1995; Sauseng et al., 2009) suggest that individual differences in theta frequencies (and gamma frequencies) could potentially be linked to individual differences in WM or STM capacity.

1.5 Brain oscillations in the context of this thesis

Given the possible relationships between frequency peaks and cognitive functions, this thesis will explore individual differences in frequencies, focusing on the question of whether they can be successfully modulated. In study 1, the question of whether individual alpha peaks can be successfully modulated will be explored in an EEG/tACS (transcranial alternating current stimulation – see next chapter for an overview of this technique) study. In chapters 5-8 the link between individual theta and gamma frequencies and working memory capacity will be explored. As with study 1, studies 2, 3 and 4 will investigate whether individual frequencies can be successfully modulated, this time focusing on theta (studies 2-3) and gamma (study 4) activity. These studies (2, 3 and 4) will also explore the causal nature of the relationship between individual theta and gamma frequencies and WM capacity.

These studies will utilise the neurostimulation technique of tACS in an attempt to cause the entrainment of endogenous brain oscillations. Therefore, chapter 2 will provide an overview of neurostimulation principles and methods followed by an overview of the principle of entrainment in chapter 3 before the first empirical study is outlined in chapter 4.

Chapter 2: Neurostimulation

2.1 Introduction

This chapter will give an overview of non-invasive brain stimulation (NIBS) techniques. These techniques can take the form of magnetic stimulation (transcranial magnetic stimulation – TMS) or electrical stimulation (transcranial electrical stimulation – tES). Whilst the studies in this thesis use a form of tES known as transcranial alternating current stimulation (tACS), this section will provide an outline and comparison of both tES and TMS as their applications overlap throughout the NIBS literature.

2.2 Overview of TMS and tES

2.2.1 TMS

TMS uses a coil to deliver a magnetic pulse to a single location on the scalp and the underlying brain regions. This pulse induces electrical current on the targeted brain region. It has been suggested that TMS supresses neural activity (e.g. Harris et al., 2008) and it has therefore been referred to as creating 'virtual lesions' (Walsh & Cowey, 1998). However, it has also been shown to enhance neural activity (e.g. Abrahamyan et al., 2015) and additionally it has been proposed that TMS disrupts neural functioning through the addition of random noise (e.g. Schwarzkopf et al., 2011). Further studies have suggested that all of these accounts of TMS activity are correct in certain circumstances and that the effects of TMS are dependant on the neural activity at the time of stimulation, hence the reason for the seemingly contrasting accounts of the effects of TMS in the literature (e.g. Perini et al., 2012; Schwarzkopf et al., 2011).

2.2.2 tES

tES techniques use two electrodes placed on the scalp. An electric current is then passed between the electrodes with some of this current passing through the underlying cortical regions. tES results in changes in the resting membrane potential of the targeted brain regions (Yavari, et al., 2018).

2.3 The safety and non-invasiveness of TMS and tES

Both TMS and tES techniques are considered safe (Antal et al., 2017), although contraindications do exist for both techniques. However, discomfort has been reported in some cases (see Fertonani et al., 2015 for a review) and in some instances tES has been known to produce temporary skin lesions (e.g. Rodriguez et al., 2014; Frank et al., 2010). Methods have been identified to reduce discomfort during tES, such as using smaller electrodes (Turi et al., 2014) and keeping the concentration of the saline solution between certain parameters (Dundas et al., 2007). Whilst both TMS and tES are referred to as non-invasive, Davis and van Koningsbruggen (2013) have argued that this is a misnomer and that NIBS techniques are in fact invasive as they involve an exogenous current being introduced into the brain with this effect then spreading to neighbouring regions.

2.4 Types of TMS and tES

2.4.1 Categories of TMS

Both TMS and tES can be subdivided further. Standard TMS emits a single pulse, whereas repetitive TMS (rTMS) emits multiple pulses, rTMS can be further subdivided into rhythmic and arrhythmic, where, as the names suggest, the trains of pulses are either rhythmic or arrhythmic. Rhythmic TMS is generally used when researching a particular frequency of brain oscillations (e.g. Romei et al., 2015) whereas single pulse TMS and arrhythmic rTMS tend to be used more in cases where the function of a particular brain region is the focus rather than a specific oscillatory rhythm (e.g. Perini et al., 2012). As mentioned above, TMS has been referred to as creating 'virtual lesions' in the brain as it can temporarily inhibit cortical activity at the focal point of the pulse, this has enabled studies to be conducted that circumnavigate some of the practical issues surrounding studies of patients with brain lesions (Walsh & Cowey, 1998).

2.4.2 Categories of tES

There are three main forms of tES: transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS). All three use the same machinery and basic mechanisms consisting of two (or more) electrodes placed at different locations on the skin (at least one of which is placed on the scalp over a cortical area). A current is then passed between the two electrodes and some of this current travels through the upper layer of the cortex, affecting the membrane potentials of the targeted area (Yavari et al., 2018).

tDCS

tDCS designates one electrode as the cathode and one as the anode. The electrical charge under the cathodal electrode is negative and the charge under the anodal electrode is positive (Woods et al., 2016) and typically, excitation is increased in areas under the anodal electrode and decreased in areas under the cathodal electrode (Antal & Herrmann, 2016).

tACS

With tACS, the direction of current flow oscillates back and forth between the two electrodes, with neither taking the role of anode or cathode. Therefore, tACS does not cause excitation under one electrode and inhibition under the other but, instead, produces an oscillating electric current beneath both electrodes (and, depending on the placement of the two electrodes relative to each other, this oscillating current can also be produced in brain regions between the two electrodes – see studies 2 and 3, chapters 6 and 7, for further discussion of this). tACS can be set to different frequencies and is often referred to by the EEG frequency band corresponding to the frequency at which the current oscillates, for example alpha tACS (αtACS; within the 8-12Hz bandwidth) or theta tACS (θtACS; within the 4-7Hz bandwidth). tACS has been shown to enhance activity around the frequency of stimulation (e.g. Helfrich et al., 2014 - also see chapter 3 on entrainment) and therefore this technique is particularly useful when studying the effects of specific oscillatory frequencies.

tRNS

With transcranial random noise stimulation (tRNS) the direction of current flow also alternates between the electrodes as with tACS, however this does not occur in a rhythmic pattern at a set frequency but rather the speed of this alternation varies across frequencies from one oscillation to the next. tRNS can be used to excite cortical areas under the electrodes (Antal & Herrmann, 2016) as with the anodal electrode in tDCS although the effects of tRNS appear to have a slower onset compared to the anodal effects from tDCS (Prichard et al., 2014).

Whilst many of the basic principles behind all forms of tES are the same, it is clear that these three tES techniques vary in their applications (see Antal & Herrmann, 2016 for an overview of the differences between these techniques). tRNS can excite cortical areas with a slower onset compared to tDCS, tDCS can both excite and inhibit specific cortical areas simultaneously and has a rapid onset relative to tRNS, and tACS can enhance cortical activity around particular frequencies (see following chapter on entrainment for a more detailed discussion about this application of tACS). It is worth noting that tDCS studies do not always have to induce both cortical excitation and cortical inhibition simultaneously; during tDCS, the cathodal (or anodal) electrode can be placed on a non-cortical area if no region of cortical inhibition (or excitation) is preferable for a particular study (see Mehta et al., 2015). Therefore, whilst the ability to induce inhibition is a possible advantage of tDCS in certain cases, tDCS can also be used when no cortical inhibition is desired.

2.5 Sham controls for TMS and tES

Sham stimulation can be used during both TMS and tES studies to control for any effects deriving from the procedures surrounding the stimulation rather than the effects of the actual stimulation. Sham stimulation consists of mimicking the procedure of active stimulation to the extent that the participant (and, ideally the experimenter too) is unaware that no active stimulation is taking place.

2.5.1 tES sham techniques

In tES studies, a slight tingling sensation under the electrodes can be experienced during the initial stages of stimulation. Generally, this sensation then disappears for the duration of the stimulation. In order to control for this, sham stimulation involves delivering an initial electric current in order to mimic any sensations that may occur during active stimulation. However, in the sham condition, the current is increased then decreased over a short time period then stimulation ceases for the remainder of the session time. This has been shown to be effective in creating double-blind scenarios where neither the participants nor experimenters were able to distinguish between sham and active stimulation (Gandiga et al., 2006; Russo et al., 2013), however there is also a suggestion that sham stimulation is marginally distinguishable from active stimulation under the anodal electrode in tDCS (Fertonani et al., 2015) although the same study found sham stimulation to be effective for tRNS, tACS and cathodal tDCS. One further potential problem with this technique that should be noted is that, whilst stimulation only occurs for a minimal amount of time during sham stimulation, stimulation does still occur briefly at the start of each session.

2.5.2 TMS sham techniques

In TMS, the emission of the magnetic pulses creates a clicking sound and sensory stimulation of the scalp. It is important for sham conditions to replicate these sensations. This is partly so that participants remain unaware they are receiving sham stimulation and also in order to control for any effects that are the result of this sensory stimulation. It has been shown that auditory stimulation from TMS can produce artifacts in EEG data, in the form of auditory evoked potentials (e.g. Nikouline et al., 1999; Tiitinen et al., 1999). This sensory stimulation may cause even more of a problem during rhythmic TMS, as entrainment of neural oscillations has been shown to occur through rhythmic sensory stimulation (e.g. Nozaradan, 2011; see also the following chapter for more information on entrainment). Therefore, sham methods which replicate these sensations have been developed.

Methods of controlling for the noise elicited by TMS

One method is to use a sham coil that replicates the noise but does not produce an electric pulse, however, one problem with this method is that it does not reproduce the tactile sensation present in active stimulation (Duecker & Sack, 2015; Rossi et al., 2007).

Methods of controlling for the tactile sensations elicited by TMS

To address the issues caused by the tactile sensations, sham stimulation techniques have been developed which deliver an electrical current to the scalp. This creates a tactile sensation similar to that of active TMS yet the electrical current used is too weak to stimulate the cortex (e.g. Mennemeier et al., 2009; Rossi et al., 2007). Whilst these studies had some success at forming a single-blind condition amongst participants not familiar with TMS ('naive' participants), participants who were experienced with TMS were able to tell the difference between these forms of sham and active TMS. Furthermore, in the study by Mennemeier et al (2009), participants not familiar with TMS were more likely to view the sham stimulation as active stimulation, an outcome which the authors acknowledge could itself produce confounds due to participant bias.

Controlling for both noise and tactile sensations elicited by TMS through the angled coil method

A common way of producing a sham condition during TMS studies, which does not require any further specialist equipment such as a sham coil, is to position the coil at an angle of 90˚ to the scalp. This replicates the noise and tactile sensation yet a large portion of the magnetic current does not stimulate the brain. However, with this method the possibility that some brain stimulation occurs cannot be ruled out (Duecker & Sack, 2015; Lisanby et al., 2001) and the tactile sensation can be weakened compared to active TMS (Lisbany et al., 2001) although it has been shown to be effective at producing a single-blind scenario (Duecker & Sack, 2015).

2.6 Parameters to consider for TMS and tES

When designing a NIBS study, there are various parameters that need to be accounted for, as these parameters can greatly affect the outcome of the results. Whilst the majority of NIBS studies remain focused on investigating a particular functional outcome, there are some studies out there which have instead focused on addressing the methodological question of how subtle variations in specific parameters can alter the effects of NIBS (for example Dowsett & Herrmann, 2016; Mazzi et al., 2017; Rothkegel et al., 2010; Schwarzkopf et al., 2011). Findings from these studies highlight how changes in parameters can affect the results of NIBS and they therefore highlight the importance of careful consideration of such parameters when designing a NIBS study. Therefore, the following section will outline the main parameters that need to be taken into account during NIBS studies.

2.6.1 Parameters to consider with TMS

The main two parameters to consider for all forms of TMS are the positioning of the coil and the intensity of the stimulation.

Positioning of the coil

As TMS provides stimulation to cortical areas directly under the coil, deciding where to position the coil is relatively straightforward once the cortical area of interest has been identified.

Intensity of stimulation

The intensity of the stimulation (i.e. the magnitude of the magnetic pulse) also needs to be considered and is not as straightforward a consideration as the location of the coil. In a TMS study by Schwarzkopf et al. (2011) they found opposite cognitive effects were produced when low intensity stimulation was compared to high intensity stimulation. It is generally considered the case that high-level stimulation impairs performance whilst low-level stimulation enhances performance (Abrahamyan et al., 2015). This highlights the importance of carefully considering the optimal intensity when planning a TMS study as a linear increase in intensity does not lead to a simple linear increase in effectiveness.

Timing of pulses

In some cases the timing of the pulses in relation to an external event or a particular endogenous oscillation is also a factor to consider.

Parameters specific to subcategories of TMS

(i) Deep TMS (dTMS)

Most TMS studies focus on surface cortical areas, however, it is possible to stimulate deeper layers of the cortex with the use of specialist TMS coils. In such instances, there are different alternatives to the standard double coil that are available for deeper stimulation during TMS and the pros and cons of these coil types need to be considered relative to the particular study being conducted as increased depth of stimulation is offset by a decrease in the focalisation of the stimulation (Lu & Ueno, 2017).

(ii) Repetitive TMS (rTMS)

In repetitive TMS studies, it is important to consider how many pulses to deliver during each application of stimulation, whether these repetitions should be delivered rhythmically or arrhythmically and, if rhythmically, which frequency would be most appropriate.

2.6.2 Parameters to consider with tES

Amplitude of stimulation

With tES techniques, the amplitude of stimulation needs to be considered. As with TMS, studies have shown different effects to occur when high and low amplitudes of currents were compared during tDCS (Batsikadze et al., 2013), suggesting that there is not a simple linear correlation between current amplitude and effectiveness of stimulation but that the interplay between stimulation amplitude and stimulation effects is far more nuanced.

Electrode size

The size of the electrodes used is a further consideration needed when using tES techniques. Smaller electrodes result in more focalised stimulation comparative to larger electrodes (Nitsche, et al., 2007). Electrode size has also been indicated as a factor affecting participant comfort during stimulation (Turi et al., 2014). (Also see below for a discussion of the additional factors to consider regarding cathodal electrode size when designing tDCS studies).

Current intensity

The size of the electrodes combined with the amplitude of stimulation determines the intensity of the stimulation (current density = current strength/electrode size; Nitsche, et al., 2007) therefore smaller electrodes result in a higher intensity of stimulation compared to larger electrodes. This is an important factor when considering the desired outcomes of the stimulation as it is not necessarily the case that more intense stimulation leads to greater effectiveness of stimulation (see Esmaeilpour et al., 2018 for a meta-analysis on the effects of current intensity in tDCS studies).

Positioning of electrodes (electrode montage)

As mentioned above, determining the ideal location of a TMS coil is relatively straightforward. However, with tES, the matter is more complicated due to the way in which the electrodes make an electrical circuit. Often in tES studies, only one cortical area is of intertest and the other electrode is placed over a non-relevant area. However, it has been shown that the positioning of this ‘other’ electrode can alter the effects of the stimulation in the area of interest (e.g. in addition to the areas directly under the electrodes receiving stimulation, the current will also pass through additional cortical areas as it travels between electrodes) (Bikson et al., 2010; Mehta et al., 2015; also see studies 2 and 3 in this thesis). Therefore, careful consideration is needed to determine the most likely routes through the brain that the current flow may take. An electric field distribution analysis (Miranda et al., 2013) provides a comprehensive probability-based profile of the amount of current delivered to all cortical areas. This allows an informed decision to be made regarding which brain areas are being stimulated. Even if such an analysis is not used, it is still important to consider the cortical areas directly under both electrodes and those that lie along the most direct path between them as these are all prone to the effects of stimulation. Perhaps most importantly, it is important to determine whether any excitation of these areas could potentially cause a confound. If so, an alternative position for one of the electrodes should be decided upon. One particular aspect of electrode placement that has received interest is whether to place both electrodes over the same hemisphere (unilaterally) or on opposite hemispheres (bilaterally) with the findings of some studies suggesting that this factor plays a role in the efficacy of stimulation (see Imburgio & Orr, 2018 for a review of some of the literature regarding this).

Considerations unique to tDCS

(i) Anodal and cathodal placement

tDCS brings unique considerations regarding the interplay between the anodal and cathodal electrodes, with the order of the anode and cathode needing to be considered in relation to whether excitation or inhibition is required over a particular region.

(ii) Methods for reducing the effect of the cathode

The relative electrode sizes of the anode and cathode is also a consideration that needs to be accounted for when designing a tDCS study (See Imburgio & Orr, 2018 for a meta-analysis of the effects of anode size in tDCS studies). In some tDCS studies, where minimal cathodal impact is required, a large cathodal electrode paired with a small anodal electrode has been used to try to reduce the effect of inhibition under the cathode (e.g. Leite, et al., 2018; Nitsche et al., 2007) (as electrode size is inversely related to intensity - see above). Whilst this may reduce the electric current under the cathode, this intervention cannot be considered to render the cathode 'redundant' as electric field distribution modelling suggests that it still effects the current flow (Bikson, et al., 2010). Other attempts to reduce the impact of the cathodal electrode include extracranial positioning (when the electrode is placed over a non-cortical area). Whilst this has been shown to produce differential effects when compared to cranial cathodal placement (consistent with the general findings from studies comparing different montages) (see the meta-analysis by Imburgio & Orr, 2018), extracranial cathode placement still impacts the pathway of the current flow across cortical regions (Bikson et al., 2010) and could possibly create greater stimulation of deeper brain areas (Noetscher, et al., 2014).

Considerations unique to tRNS

(i) Frequency range

The range of frequencies that the repetitions will span is an additional factor to consider when designing tRNS studies.

Considerations unique to tACS

(i) Frequency

The frequency of stimulation is an important factor in the majority of tACS studies. Whilst it is often known which frequency band is of interest to a particular study, the frequency of stimulation within a band that is optimal for a specific experiment can be more nuanced. As mentioned in chapter 1, frequency peaks within a band can increase or decrease depending on the type of cognitive task being carried out (e.g. Haegens et al., 2014), this can be used to guide experimenters towards the most appropriate stimulation frequency to use. Another factor to consider is whether or not to tailor the stimulation frequency to match oscillatory activity to individual participants (e.g. stimulating at an individual's peak alpha frequency, IAF, as opposed to an average peak alpha frequency of 10Hz). In the given example IAF would produce more tailored stimulation yet a uniform stimulation frequency of 10Hz would be more practical and would not require any physiological recordings (e.g. EEG or MEG) to take place prior to stimulation.

(ii) Waveform shape

Waveform shape is a factor to consider when delivering tACS. The majority of studies to date have used sinusoidal waveforms by default, however, it has been suggested that a more tailored approach to waveform shape is preferable as in many cases sinusoidal stimulation may not be optimal (Dowsett & Herrmann, 2016). This is a relatively new area of investigation and further studies of this parameter, particularly those conducting systematic comparisons of waveform shapes as with Dowsett and Herrmann (2016), may help to guide future studies in rhythmic stimulation.

(iii) Desynchronisation across a network

As mentioned above, electrode montage is an important consideration for all tES techniques. However, an additional factor to be considered in the case of tACS, is whether the two electrodes cover two different areas of a network that could play a role in the function being investigated. Due to the nature of tACS, the sites under the two electrodes are stimulated at different phases relative to each other (180˚ out of synch). If these two sites encompass different parts of the same network then desynchronisation across that network could be triggered, potentially leading to confounding effects (see studies 2 and 3 for further discussion and an empirical investigation of this).

2.7 Applications of TMS and tES

2.7.1 Causal investigations

As neurostimulation can non-invasively and temporarily alter brain activity, it can be particularly useful for investigating causality rather than just correlations in the relationships between brain activity and cognitive functions (Yavari et al., 2018). All NIBS techniques lend themselves to the study of the relationships between specific cortical regions and behavioural and cognitive functions (e.g. Prichard et al., 2014; Abrahamyan et al., 2011) and, additionally, rhythmic TMS and tACS can be useful when studying the causal effect of neural oscillations at particular frequencies or within particular frequency ranges (e.g. Hanslmayr et al., 2014; Pollok et al., 2015).

2.7.2 Therapies

NIBS has shown therapeutic potential when areas linked to certain pathologies are stimulated (e.g. Ficek et al., 2018; Mori et al., 2010) and when stimulation occurs at frequencies that have been implicated in pathologies (e.g. Naros & Gharabaghi, 2017). This will be looked at further in the following section.

Considerations regarding therapeutic applications of NIBS

(i) After effects of NIBS

For NIBS techniques to be used successfully in a therapeutic context it would be extremely advantageous if the beneficial effects of the stimulation continued after the stimulation had ceased. Studies investigating single session stimulation have generally found after-effects to either only last for the length of stimulation (e.g. Naros & Gharabaghi, 2017) or to last for an amount of time that would not be particularly useful when applied therapeutically for long-term conditions (e.g. an after effect lasting no longer than 70 minutes, Kasten et al., 2016) (in a review of the after-effects of NIBS, Veniero et al., 2015, found no consistent pattern across studies). However, studies investigating the effects of multiple sessions of NIBS have shown that regularly repeated sessions can produce effects that last for longer than the duration of the stimulation session to a level that can be beneficial to patients (e.g. Cappelletti et al., 2013; Klimke et al., 2016; Mori et al., 2010). Major depressive disorder is a particularly promising candidate for a stimulation treatable pathology with several studies finding promising results (e.g. Donse et al., 2018; Pridmore et al., 2018; see Perera et al., 2016 for a review of TMS as a treatment for major depressive disorder). Additionally, in the study by Pridmore et al. (2018) there was evidence that monthly 'maintenance' sessions of TMS could help reduce relapse rates in major depressive disorder after remission had been achieved from a successful initial course of TMS, further supporting the idea that regular sessions of NIBS can produce benefits that outlast the stimulation session.

(ii) Comparison of TMS and tES for therapeutic uses

When considering therapeutic applications, tES has some practical advantages over TMS. tES is cheaper, more portable and can be self administered relatively safely comparative to TMS, giving it three major advantages for potential clinical use. Martens et al., (2018) conducted a study where tES was applied to patients by their caregivers in the patients' own homes (e.g. Martens et al., 2018), this would not have been practical with current TMS technology. Because of these benefits of tES, if a potentially therapeutic effect is found during a TMS study, it may be useful to investigate whether a similar effect can be found using tES.

2.8 Simultaneous neuroimaging with NIBS

When concurrent neuroimaging recordings (such as fMRI, EEG or MEG) are required alongside NIBS, the imaging data can become contaminated by stimulation artifacts (see figure 3). Different combinations of NIBS and imaging techniques each come with unique challenges for noise reduction. However, relatively recent advancements in noise reducing algorithms continue to make it more feasible to combine such techniques. Combining EEG and single pulse TMS is relatively straightforward as artifacts produced by the TMS pulse are not ongoing and are therefore easily removed from the continuous EEG data. Combining EEG and tES remains a more complicated technique compared to simultaneous EEG/TMS due to the ongoing nature of the tES activity. However, recent advancements in noise reducing algorithms have made simultaneous EEG/tES possible. In a study investigating alpha activity and visual target detection, Helfrich et al. (2014) used concurrent tACS and EEG to demonstrate that tACS at 10Hz increased overall alpha power and, in a study designed to demonstrate the feasibility of combining these techniques, Witkowski et al. (2016) successfully removed tACS artifacts from MEG data.[4] However, there have been criticisms of the algorithms used when separating tES artefact from EEG data (e.g. Noury et al., 2016; Noury & Siegel, 2017).

fMRI has also been successfully combined with NIBS. TMS and concurrent fMRI have been successfully combined to study, for example, the motor cortex (Bohning et al., 1998; Roberts et al., 1997) and visual cortex (Ruff et al., 2006) (also see Ruff et al., 2009 for an overview of combining TMS and fMRI) and tES has been combined to investigate speech facilitation (e.g. Holland et al., 2011) also see Saoite et al., 2013, for an overview of fMRI with the different forms of tES).

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[4] For further discussion of the additional issues arise when combining rhythmic NIBS such as tACS with EEG and MEG see chapter 3, section 3.5.

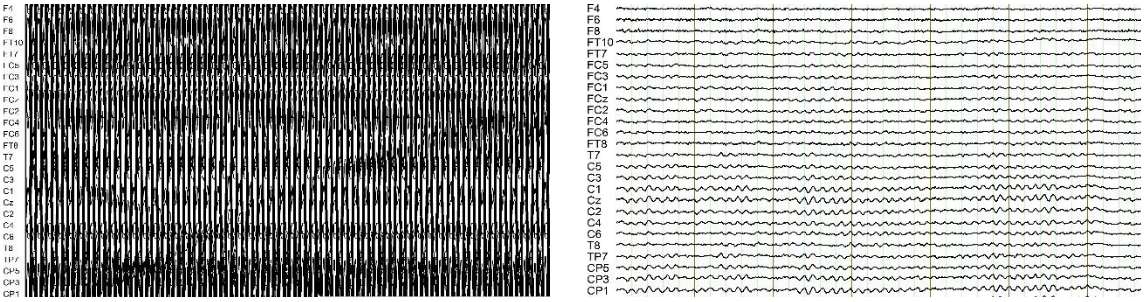

Figure 3. The image on the left shows raw resting EEG data during concurrent tACS; the artifacts from electrical noise can be seen clearly. The image on the right shows normal raw resting EEG data for comparison.

2.9 Entrainment through rhythmic neurostimulation

One particular outcome that can be achieved through rhythmic neurostimulation is the modulation of oscillatory activity around the frequency of stimulation through the entrainment of neural oscillations (e.g. Helfrich et al., 2014) (although entrainment can also be achieved through sensory stimulation, e.g. Notbohm et al., 2016). This particular application of rhythmic neurostimulation (specifically, tACS) forms the basis of the studies throughout this thesis and therefore an overview of entrainment will be given in the following chapter.

Chapter 3: Entrainment

3.1 Overview of entrainment

Entrainment of brain oscillations occurs when neural oscillations become synchronised with the rhythm of an external oscillator (Thut et al., 2012). The endogenous neural rhythm shifts towards the exogenous rhythm until it is phase-locked to it (see figure 4). The entrainment of neural oscillations has been likened to the analogous entrainment of circadian rhythms that occurs when a person moves to a different time zone and their sleep-wake patterns shift until they are synchronised with the new rhythm of day and night (Thut et al., 2012).

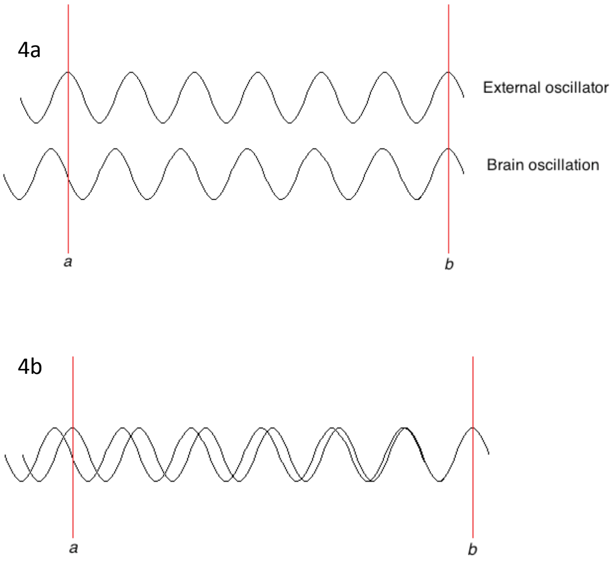

Fig 4a. A brain oscillation becoming synchronised to an external oscillator. At time point *a* the two oscillations are out of phase, yet by time point *b* the brain oscillation has altered to become synchronised with the external oscillator.

4b. The two oscillations are superimposed on each other for added clarity.

When multiple neural oscillations become synchronised to an external oscillator through entrainment it logically follows that these endogenous oscillations are, as a result, synchronised with each other. Consequently, an increase in phase synchronicity at the frequency of entrainment occurs. An increase in the synchronisation of oscillatory activity at a particular frequency results in an increase in power (amplitude) at that frequency (Nunez & Srinivasan, 2006). Therefore, as entrainment produces synchronicity of endogenous oscillations to an exogenous oscillator, a logical outcome of entrainment would be an increase in power (indicating an increase in synchronicity) at the frequency of the exogenous oscillator. Power increases due to entrainment will be discussed in more detail below.

3.2 Types of external oscillators

3.2.1 Sensory stimuli

Different types of external oscillators can be used for the entrainment of brain oscillations. One form of external oscillator takes the form of sensory stimuli presented rhythmically at a consistent frequency. Studies have demonstrated that entrainment of neural oscillations can result from rhythmically presented visual stimuli (e.g. Mathewson et al., 2012; Zauner et al., 2012). Similarly, it has been demonstrated that auditory stimuli can also entrain neural oscillations (e.g. Nozaradan et al., 2011). However, there has been some debate regarding whether what appears to be entrainment through sensory stimuli can instead be explained by repeated event-related responses (e.g. Capilla et al., 2011; Keitel et al., 2014; Lithari et al., 2016), although see (Notbohm et al., 2016) for the opposing view that entrainment does take place. A more recent paper by Keitel et al., (2018) posits that both interpretations are at work simultaneously.

3.2.2 Non-invasive brain stimulation (NIBS)

Another form of external oscillator that has been used for neural entrainment is rhythmic, non-invasive brain stimulation (NIBS). The two main categories of rhythmic NIBS used for neural entrainment use either magnetic pulses, such as with rhythmic TMS, or electrical currents, such as with tACS, (see previous chapter for an overview of both rhythmic TMS and tACS) and studies have demonstrated successful neural entrainment with these techniques (e.g. Helfrich et al., 2014; Romei et al., 2016). This thesis will largely focus on the use of NIBS as an external oscillator, with the studies in this thesis utilising the technique of tACS.

3.3 Outcomes of entrainment

3.3.1 Power increases

To date, the majority of entrainment studies have investigated stimulation delivered at peak frequencies within the given frequency band of interest. These have been either tailored to the individual participant's peak frequency of interest (e.g. Romei et al., 2016) or delivered at an average (typical) peak frequency (e.g. Helfrich et al., 2014). Such studies have reported subsequent power increases within the stimulated frequency range (see below for further examples of studies). As mentioned above, such increases in power suggest that more phase-locking has taken place and it can be inferred that this is due to the brain oscillations becoming phase-locked to the external oscillator (the fact that they are then synchronised with each other can almost be considered a by-product of this phase-locking to the external oscillator, a by-product that nonetheless is extremely important). Entrainment studies have demonstrated increases in power around the frequency of stimulation through both physiological measures and behavioural findings and these will now be looked at below.

Behavioural evidence for power increases

Behavioural studies have demonstrated the occurrence of entrainment by testing cognitive functions that have been previously associated with an increase in power around particular frequencies or frequency bands. One example is the study by Romei et al., (2010) who found behavioural evidence for the entrainment of alpha oscillations through the use of alpha transcranial magnetic stimulation (αTMS). By delivering TMS pulses rhythmically at alpha frequency during a visual detection task, Romei and colleagues demonstrated that αTMS delivered to occipital or parietal areas caused changes in visual detection to both the contralateral and ipsilateral visual fields. These changes were in line with effects that had previously been shown to correlate with increased alpha power (Romei et al., 2010). Transcranial alternating current stimulation (tACS) has also been shown to produce behavioural effects consistent with the entrainment hypothesis. In one study, Pogosyan et al. (2009) used tACS delivered at a beta frequency over the motor cortex and found that it slowed voluntary motor responses in the contralateral hand, an effect associated with an increase in beta power. In a study by Jausovec & Jausovec (2014), theta tACS applied over the left parietal region led to an improvement in working memory performance, an effect which has been linked to an increase in theta power (e.g. Raghavachari et al., 2006). Alpha stimulation has also been shown to improve short-term memory capacity in line with a predicted increase in alpha power (Sauseng et al., 2009).

Physiological evidence for power increases

Physiological studies have provided further support for the entrainment hypothesis through the observation of power increases around the stimulation frequency. Such studies have been conducted for rhythmic TMS (e.g. Thut et al., 2011) and tACS (e.g. Helfrich et al., 2014; Neuling et al., 2012; Zaehle et al., 2010). These studies have demonstrated power increases around the stimulation frequency when the stimulation frequency was kept constant across participants (as in the studies by Helfrich et al., 2014 and Neuling et al., 2012, which used a stimulation frequency of 10Hz throughout) and also an increase in power around the stimulation frequency when the stimulation was matched to the participants' individual alpha frequencies (IAF) (as was the case in the study by Thut et al., 2011).

The combination of physiological and behavioural evidence for power increases

As illustrated above, the findings from physiological and behavioural studies have complemented each other. Findings from physiological studies have provided direct evidence that the introduction of an external oscillator can cause a measurable increase in power of brain oscillations. Whilst findings from behavioural studies are unable to provide direct evidence for changes on a physiological level, they have the benefit of being able to demonstrate that stimulation can produce effects that are robust enough to be seen on a behavioural level. This is particularly important when considering the potential usefulness of entrainment. Evidence that entrainment can cause behavioural and cognitive changes due to power increases can be useful when deciding on some of the potential applications for this area of neurostimulation (for example, treatments for pathologies that are associated with a decrease in power).

3.3.2 Frequency shifts

The majority of studies investigating entrainment, such as those mentioned above, have focused on enhancing the power at pre-existing average frequencies/frequency peaks. However, another potential application of entrainment is the modulation of frequency peaks using off-peak entrainment. During the process of off-peak entrainment, an external oscillator oscillates at a frequency that does not match the dominant (or peak) frequency of the endogenous oscillations. Any subsequent entrainment would entail the endogenous oscillations altering in frequency to become synchronised to the exogenous oscillator, as opposed to just undergoing a shift in phase. As a result of the endogenous oscillations becoming entrained (synchronised) to the frequency of the exogenous oscillator, a large number of neural oscillations would be synchronised with each other at this new frequency, leading to an increase of power around this specific frequency point. Therefore, by increasing the power at a new frequency, this technique could potentially be used to alter the peak frequency. For example, Cecere et al. (2015) delivered tACS at peak and off-peak (both high and low) alpha frequencies during the sound induced flash illusion task (an audio-visual illusion task where individual differences in performance are linked to individual differences in alpha frequency peaks). Performance on the task across stimulation conditions reflected changes in alpha peaks in the direction of stimulation, suggesting that frequency shifts had occurred through off-peak entrainment. This thesis will look further at the possibility of using stimulation delivered at off-peak frequencies in order to shift frequency peaks through increasing off-peak power. This is explored in the studies of this thesis both in terms of physiological changes (study 1) and in behavioural changes that occur as a result of off-peak entrainment (studies 2, 3 and 4).

Whilst off-peak entrainment is relatively new compared to entrainment delivered at peak frequencies, an overview of the current literature surrounding this application of entrainment will now follow.

3.4 Overview of the current literature regarding off-peak entrainment

Prior to any physiological studies directly researching off-peak entrainment, there had been some suggestions that frequency shifts could occur when stimulation was applied at off-peak frequencies. Helfrich et al. (2014) applied tACS at 10Hz for all participants in an entrainment study. The original aim of this study was to enhance alpha power and this effect was found. However, additionally it was noted that the range of individual alpha frequency peaks during stimulation was smaller and shifted towards 10Hz when compared to the range of IAFs prior to stimulation. As acknowledged by the authors of the paper, this suggested that endogenous neural oscillations had shifted towards the frequency of stimulation. However, in a study by Vossen et al., (2015) investigating the after-effects of tACS, it was found that after-effects of off-peak rhythmic stimulation within the alpha band did not take the form of an increase of power around the stimulation frequency but instead took the form of a power increase at the original individual alpha frequency (IAF). In this study, off-peak stimulation occurred as a by-product of fluctuating inter-individual IAFs across multiple sessions (with the IAF from the first session being used across all sessions) and the subsequent analysis of off-peak stimulation was carried out in light of this. Therefore, as with the study by Helfrich et al., (2014), the modification of individual frequency peaks was not the main aim of this study and systematic and consistent off-peak stimulation across participants was not carried out.

The studies in this thesis aimed to address the absence of a systematic investigation into the possibility of frequency modulation through off-peak entrainment in the entrainment literature. Since then, studies have started investigating this area directly. In a behavioural study specifically designed to investigate off-peak entrainment, Vosskuhl et al. (2015) demonstrated that theta tACS delivered below the individual theta peak frequencies (ITF) of participants increased STM capacity. An increase in STM load has been linked to lower theta peaks (e.g. Axmacher et al., 2010), and the causal nature of this relationship found in the study by Vosskuhl and colleagues is in line with the theta-gamma phase coupling theory of STM capacity (Lisman and Idiart, 1995) (see chapter 5, section 5.2.1, for more discussion of this theory). Therefore, these findings provided behavioural support that theta frequencies had been lowered during stimulation. However, the off-peak stimulation was compared to a control condition with no active stimulation, therefore, the possibility that any effects found were the result of a power increase in the theta band from theta stimulation in general could not be ruled out. In a tACS study by Cecere et al. (2015), entrainment through stimulation delivered at peak alpha frequencies (IAF) was investigated alongside stimulation at 2Hz above and 2Hz below IAF. Behavioural effects in terms of susceptibility to the visual flash-beep illusion were in line with frequency shifts in IAF in the direction of the stimulation frequency. More recently, a tACS study by Minami and Amamo (2017) provided both behavioural and physiological evidence that off-peak alpha stimulation can produce shifts in IAF, again, both increasing and reducing the original IAF in line with the off-peak direction of the stimulation frequency. The studies in this thesis add to this relatively new area of literature surrounding the off-peak entrainment of neural oscillations.

3.5 Duration of entrainment after stimulation has ceased

How long the effects of entrainment can last after the exogenous oscillator has stopped is a particularly important question when considering whether entrainment can be used therapeutically (Veniero et al., 2015). It also has practical implications when investigating the physiological effects of entrainment. There are certain practical constraints involved when recording brain oscillations alongside an exogenous oscillator, as the oscillator creates rhythmic noise. This is particularly troublesome for entrainment studies as, generally, the exogenous oscillator is oscillating at the same frequency as the neural oscillations of interest. The problem is exacerbated when the stimulation and physiological recordings are of the same domain, for example they are both electrical in nature (i.e. a tES and EEG combination). As a result of these practical constraints, many entrainment studies take physiological recordings just before and just after the application of rhythmic stimulation and compare the two recordings in order to ascertain whether any effects took place (e.g. Vosskuhl et al., 2014; Zaehle et al., 2010); although see Thut et al., (2011) for an example of simultaneous TMS and EEG and Helfrich et al. (2014) for an example of EEG being successfully recorded during tACS. The use of pre and post stimulation physiological recordings as a way for testing if entrainment occurred relies on it being the case that the effects of entrainment can outlast the stimulation period (in order for them to be apparent in any subsequent physiological recordings). Additionally, if any effects are found that outlast stimulation, there is still the question of how representative these effects are to the physiological processes that occur during the entrainment. This section will now explore some studies that have investigated this question.

Hanslmayr et al., (2014) conducted a study delivering beta TMS during a memory task paradigm. Behavioural results from the memory task that occurred during stimulation were indicative of an increase in beta power and offline EEG recordings taken after stimulation had stopped showed that an increase in beta power outlasted the stimulation by 1.5 seconds. This was referred to as an entrainment 'echo'.

Whilst the after-effects found by Hanslmayr and colleagues were extremely transient, Kasten et al., (2016) found after-effects of alpha tACS (in terms of an increase in alpha power) that lasted up to 70 minutes comparative to sham stimulation. (It is worth noting that in this study the alpha power in the active stimulation group did not return to pre stimulation levels after 70 minutes, but rather, the alpha power in the sham stimulation control group reached the same levels as the active stimulation group after this amount of time).

Veniero et al., (2015) reviewed several studies that showed lasting effects after rhythmic stimulation had ceased. However, they concluded that these effects were distinct from the online effects of stimulation. This review highlighted a limitation of physiological studies that compare pre and post physiological recordings as an alternative to online recordings; whilst such studies are able to shed light on any potential after effects, their findings cannot necessarily be extrapolated to online effects of stimulation.

3.6 Unanswered questions regarding neural entrainment

As off-peak neural entrainment (and neural entrainment in general to a lesser extent) is a relatively new field of study, there are many unanswered questions regarding the mechanisms behind it. For entrainment to occur, the external oscillator must be set to a frequency that falls within the parameters of the system to be entrained (i.e. the internal hardware must exist that enables the system to oscillate at that particular frequency) (Romei et al. 2016). However, to what extent this is the case regarding brain oscillations is relatively unexplored at present. There are many outstanding questions, covering both the practical applications of entrainment and the physical mechanisms behind it. For example, the degree to which the frequency of an external oscillator can deviate from the naturally preferred frequency of the targeted neural oscillations, before entrainment is no longer possible, is relatively unexplored. Whilst the answer to this question would be expected to depend on many factors including the target frequency band and brain region, this does not detract from it being a question worth exploring. Another question worth investigating is whether entrainment causes underlying neural systems to alter in frequency or whether it instead works by causing neural systems that pre-existed at that frequency to become more dominant.

Whilst it is clear that any frequency shifts occurring from off-peak entrainment would be a logical continuation of the power enhancements found when stimulation is delivered at pre-existing frequency peaks, some questions arise regarding the exact nature any frequency shifts may take. For instance, if the peak moves, does the overall power of the new peak remain the same as the original peak prior to stimulation or is a general enhancement of power also found through off-peak stimulation? (This also leads to an additional question of which parameters can affect this). Alternatively, does the overall power in the stimulated frequency band reduce, indicating the possibility of a desynchronisation between those oscillations that were effectively entrained and those that were not? Answers to these questions (through physiological recordings) would provide some insight into the mechanisms behind off-peak entrainment.

Studies systematically testing varying parameters and further research into the underlying mechanisms of neural entrainment may help to answer questions such as these and could help guide future empirical research into neural entrainment.

3.7 Potential applications of neural entrainment

As some neuropathologies have been linked to changes in both power and frequency peaks within particular frequency ranges (e.g. Eusebio and Brown, 2009; Dickinson et al., 2018) there is the possibility that modulation of power or frequency in such cases could be of therapeutic benefit. Therefore, further investigation into neurostimulation techniques that can alter this activity could potentially allow for new therapeutic techniques to be developed based around neural entrainment.

This thesis predominantly aims to explore the potential applications of tACS when applied at off-peak frequencies to see if it can modify individual frequency peaks. If such modification is possible, off-peak tACS could potentially be used therapeutically in the future for neuropathologies that result from alterations in frequency peaks.

3.8 Entrainment in the context of this thesis

In summary, this thesis aims to explore the effects of entrainment through off-peak stimulation further. This is done through a series of empirical studies, with a particular focus on the whether off-peak entrainment can successfully alter endogenous frequency peaks.

3.8.1 Study 1

The first study will investigate the physiological after-effects of off-peak tACS and the specific ways in which endogenous frequency peaks may be altered in the post stimulation EEG. Thus, study 1 attempts to address some of the questions highlighted in section 3.6 as far as possible given the technical constraints of the study. However, as mentioned in this chapter and discussed further in study 1, potential conclusions to these questions are limited somewhat by the difficulties of concurrent tACS and EEG.

3.8.2 Studies 2, 3 and 4

Studies 2, 3 and 4 further investigate the modulation of frequency peaks through off-peak entrainment. These studies address some of the practical issues arising from the use of off-line physiological measurements in study 1 by using on-line behavioural indicators of frequency modulation. The findings from these studies highlight a practical application of off-peak entrainment in relation to working memory. Additionally, comparisons of the results from these three related studies (particularly in the case of studies 2 and 3) will also illuminate the impact of different parameters in tACS entrainment studies, providing insights into the methodological considerations that play an important role when designing such studies.

The first of these empirical studies will be focused on in the following chapter.

Chapter 4: Study 1:

The physiological effects of off-peak tACS

4.1 Introduction

As mentioned in the previous chapter, most entrainment studies to date have focused on entrainment at peak frequencies. The literature demonstrates power increases through stimulation at both individually tailored peaks and at average peaks and this provides a promising picture that entrainment can occur in this form (e.g. Helfrich et al., 2014; Thut et al., 2011). However, there is currently a less clear picture surrounding frequency modulation through off-peak entrainment due to the fact that fewer studies have been conducted in this area. Some findings have been anecdotal observations made from the use of stimulation at average frequencies (e.g. Helfrich et al., 2014) however, more recently, studies have set out to investigate off-peak entrainment, (e.g. Cecere et al., 2015; Minami & Amamo, 2017; Vosskuhl et al. 2015). The findings from these studies suggest that it may be possible to shift individual frequency peaks through off-peak entrainment. This study aimed to expand on the current literature in this relatively new area by providing a systematic investigation into the physiological effects of off-peak alpha tACS. This was achieved by stimulating at frequencies both above and below individual peak frequencies (IAF) (with the two active conditions providing a control against any general effect of stimulation) along with a sham stimulation condition to control against any effects caused by factors other than stimulation. The alpha frequency band was chosen as it is often found to provide clear peaks (Kropotov,

2009) and because many previous entrainment studies have focused on alpha oscillations with successful results (e.g. Helfrich et al., 2014; Mathewson et al., 2012; Notbohm et al., 2016; Thut et al., 2011).

Specifically, tACS was delivered at 2HZ above and 2Hz below IAF, and EEG recordings immediately prior to and immediately after stimulation were compared. The aim was to see whether alpha stimulation at 2Hz off-peak was able to shift an individual's peak alpha frequency and therefore produce physiologically recordable differences in IAF. As the primary aim of this study was to investigate the potential methodology, the measures used were purely physiological with no cognitive functions investigated. It was anticipated that rhythmic alpha stimulation delivered at frequencies below and above the IAF (off-peak stimulation) may be able to shift the IAF in the direction of the frequency of stimulation (i.e. make the IAF lower and higher respectively) and that such changes may be detectable on an EEG recorded immediately following the stimulation in the form of after-effects (e.g. Kasten et al., 2016).

4.2 Method

4.2.1 Participants

19 participants were originally recruited. One did not return for the second session, therefore their data were discounted. A further 3 participants were excluded from analysis, two due to excessive EEG artifacts and one due to them having two distinct alpha peaks, which could have potentially caused noise during the subsequent analyses.

This resulted in the data from 15 participants being used in the final analyses (10 female, mean age 23.1 years, SD ± 5.2). All participants were 18 or older and were safety screened for tACS. Participants gave written informed consent before taking part. The study was approved by the local ethics committee.

4.2.2 tACS stimulation

Electrical stimulation was delivered through two 3x3cm rubber electrodes placed inside saline soaked sponges (100mM solution) attached to an EasyCap EEG cap (Brain Vision UK, London) using a NeuroConn electrical stimulation device (neuroCare Group GmbH). 3x3 cm electrodes were used as they were considered large enough to provide adequate stimulation whilst being small enough to minimise interference with the EEG electrodes (only the EEG electrodes P4 and Cz were covered by the tACS electrodes). The tACS electrode sites corresponded to electrodes P4 and Cz using the 10-20 system (see Klem et al., 1999). P4 was chosen as a stimulation site, with the immediately surrounding electrodes as the area of interest in order to stimulate the occipital-parietal alpha rhythm on the right hemisphere, as this rhythm usually displays clear alpha peaks (Haegens et al., 2014; Tuladhar et al., 2007). Cz was used as a neutral area to place the other electrode in line with previous studies in the tES literature (e.g. Kasten & Herrmann, 2017; Neubauer et al., 2017; Ruhnau et al., 2016; Sung & Gordon, 2018).

Stimulation was delivered at 2000μA unless this caused visual disturbances in which case a lower current that did not produce visual disturbances was used (this occurred for one participant who, as a result, received stimulation at 1500μA for all three conditions). The intensity of 2000μA was chosen as a standard stimulation intensity (e.g. Mehta et al., 2015) which was likely to be effective yet unlikely to cause discomfort or be distinguishable from sham stimulation. In the active conditions (IAF+2Hz and IAF–2Hz), stimulation was delivered for 20 minutes. The 20 minutes were inclusive of a ramping up period that took place for r cycles where r = 10 x stimulation frequency. In the sham condition, the stimulation lasted for c cycles where c = 30 x stimulation frequency and was inclusive of ramping up and ramping down periods (each lasting for r cycles). For the remainder of the 20 minutes in the sham condition, no stimulation was given. Throughout all stimulation sessions, impedance levels were kept below 5kΩ.

4.2.3 EEG recording

EEG data was recorded from 64 electrode sites using an EasyCap EEG cap filled with ECI electro-gel conductive gel. Impedance levels were kept below 10μ. Data was referenced online to FPz. Throughout all EEG recording sessions participants looked at a white fixation cross (15x15 mm) positioned centrally on a black background on a screen measuring 1025 x 570 mm. As offline aftereffects of entrainment have been shown to often be relatively short-lived (e.g. Chaieb et al., 2014, also see Veniero et al., 2015) a short duration for the EEG recording was needed to ensure that the EEG data was still relevant for investigating any effects of the stimulation. However, a long enough recording was necessary in order to provide enough data for a reliable average. Therefore 5 minutes of recording was decided upon as a compromise between these two considerations.

4.2.4 Order of sessions

Each participant took part in three stimulation conditions (sham, IAF+2Hz and IAF-2Hz) that took place over two experimental sessions. In one session (the short session) participants received one active stimulation session. In the other session (the long session) participants received sham stimulation in the first half of the experiment followed by active stimulation in the second half of the experiment. Participants were unaware of which stimulation session was sham. For the sham condition, the stimulation frequency throughout the ramping up and down period at the start was the same as the stimulation frequency for the active tACS session that was conducted in the second half of the same experimental session. The order of active stimulation conditions and the order of the long and short sessions were counterbalanced across participants. Whether the IAF+2Hz or IAF-2Hz condition occurred during the long or short session was also counterbalanced across participants.

4.2.5 Experiment procedure

Short session

A resting EEG was recorded for 5 minutes whilst participants looked at the fixation cross. The EEG data was re-referenced to the common average reference, and the sampling rate was changed to 256Hz. The data was then segmented into 5 second epochs (a standard epoch length, e.g. Azami et al., 2017; Saltuklaroglu et al., 2017) and epochs with excessive artifacts were rejected through visual inspection. A fast Fourier transform (FFT) was then applied to the cleaned data (resolution = 0.125Hz) and the alpha peak from the electrodes of interest (P2, P6, PO4, CP4, P4) was determined to identify the individual alpha frequency (IAF) for the participant. (See Data Preparation section for a more detailed account of this process).

Participants then received 20 minutes of active stimulation with a stimulation frequency of either IAF+2HZ or IAF–2Hz. During this 20 minute period participants remained seated in front of the fixation cross although they were instructed that they did not have to remain focused on the cross in order to minimise discomfort, eye fatigue and drowsiness. After the stimulation session, a further 5 minutes of resting EEG was recorded.

Long session

For the long experimental sessions, an initial 5 minutes of EEG recording was conducted as with the short session and the IAF was identified in the same manner. Participants then received 20 minutes of sham stimulation followed by 5 minutes of resting EEG. This was followed by a ten minute rest break.

After the rest break, another 5 minute EEG recording was conducted and again an IAF was identified through an FFT. 20 minutes of active stimulation then followed with the stimulation frequency set to either IAF+2Hz or IAF–2Hz depending on the condition. After the stimulation session a further 5 minutes of resting EEG was recorded.

At the end of each session participants were thanked and compensated with either course credits or monetary reimbursement. At the end of the second session participants were debriefed and invited to ask any questions.

4.2.6 Data preparation

All raw EEG data was pre-processed prior to analysis using Brain Vision Analyzer 2.0. Firstly, the raw EEG data was re-referenced to the average reference and the data was down-sampled to a new sampling rate of 256Hz (spline interpolation was used to smooth data in case of spikes). Data was then segmented into 5 second epochs and epochs with excessive artifacts were rejected manually through visual inspection. A Fast Fourier Transform (FFT) was applied with a resolution of 0.125Hz and the FFT data was then averaged.

The averaged FFT data for each EEG recording was then exported into a data file showing the power at each electrode recording across frequency points at intervals of 0.125Hz.

Whilst the initial peaks had been calculated across electrodes P2, P4, P6, PO4 and CP4, subsequent analyses of the data used the three electrodes P2, P6 and PO4 as the areas of interest. P4 was rejected due to its unreliability as one of the tACS electrodes was placed underneath it. CP4 was rejected as it was later decided that the position was too anterior to be considered an area of interest. These decisions were made prior to inspection of the data.

4.3 Results

The analyses of the exported data aimed to answer four main questions: 1) Did the frequency of the IAF change after stimulation? 2) Did the power at the frequency of stimulation change after stimulation? 3) Did the power at the original IAF peak change after stimulation? 4) Did the overall power across the alpha band change after stimulation?

The appropriate analyses were conducted and results for each of these questions will now be outlined in turn.

4.3.1 Question 1: Did the frequency of the IAF change after active stimulation?

Weighted averages across electrodes P2, P6 and PO4 were calculated using the formula *power (µv) x frequency (Hz)*. This formula was applied to frequency points at 0.125Hz intervals (see method section) for frequencies between 7-14Hz. The alpha range of 7-14Hz was used as this is the largest of the commonly used alpha ranges (compared to, for example, 8-12Hz) and the nature of this experimental manipulation meant that the frequencies of interest were likely to extend to the extremities of the alpha range. These weighted values for each of the frequency points were then averaged across the entire alpha range to calculate the weighted IAF.

The weighted IAFs from the pre-stimulation sessions were then subtracted from the weighted IAFs from their respective post-stimulation sessions to produce an IAF difference value for each stimulation condition (plus, minus and sham) for each participant. A repeated measures ANOVA was then conducted on these values with the stimulation condition being the within-subjects factor and the IAF difference value being the dependent variable. No significant effect of stimulation condition was found ($F(2,28) = 0.35$, $p = 0.70$; $\eta_p^2 = 0.025$). Descriptive statistics were also calculated (table 1).

	Minus	Sham	Plus
Mean	-0.052	-0.011	-0.031
Std.Dev.	0.13	0.12	0.12

Table 1. Means and standard deviations for each stimulation group

As can be seen from the ANOVA results, there were no significant differences between any of the conditions. This suggests that any changes in IAF after stimulation were not significantly different for either of the active conditions compared to the sham condition. Any potential changes in IAF were therefore unlikely to be due to the stimulation.

4.3.2 Question 2: Did the power at the frequency of stimulation change after stimulation?

For the EEG recordings of the plus and minus condition sessions, the power at the stimulation frequency was averaged across electrode sites P2, P6 and PO4 for both pre and post stimulation EEG recordings. For the sham condition sessions, the power at both 2Hz above and 2Hz below the pre sham IAF was calculated for both the pre and post sham stimulation EEG recordings. This was done to create an appropriate control comparison for both the plus and minus stimulation conditions despite there not being an actual frequency of stimulation in the sham condition. The power values for the pre-stimulation EEG recordings were subtracted from the power values of the relative post-stimulation recordings. This resulted in four difference-in-power values (Sham-plus, Sham-minus, Active-plus, Active-minus). Paired values t-tests were carried out with the following pairings: Active-plus/Sham-plus and Active-minus/Sham-minus. The values of these t-tests are displayed in table 2a. Mean values and standard deviations are displayed in table 2b.

	t	*p*	*r*
Active-plus/Sham-plus	1.6	0.13	0.38
Active-minus/Sham-minus	1.3	0.21	0.32

Table 2a. Paired T-test results for differences in power values

	Mean	Std.Dev.
Active plus	0.0080	0.15
Sham plus	-0.030	0.10
Active minus	0.053	0.093
Sham minus	0.016	0.082

Table 2b. Descriptive statistics for differences in power values

As is evident from table 2a, no comparisons reached significance. Therefore, the difference in power at the stimulation frequency in the before and after stimulation EEG recordings did not significantly differ between the active stimulation and sham stimulation groups.

4.3.3 Question 3: Did the power at the original IAF peak change after stimulation?

The power at the original IAF was determined for each EEG recording. The original IAF refers to the IAF calculated in the pre-stimulation EEG recordings for each pair of pre and post EEG recordings and from herein will be referred to as oIAF.

First a simple, non-sham-corrected analysis was conducted to see if there was a significant change in power for each of the stimulation conditions. Paired values t-tests were carried out comparing the power at the oIAF during the post stimulation EEG recordings with the power at the oIAF during the respective pre stimulation EEG recordings. This resulted in three t-test comparisons: Sham post/Sham pre, Plus post/plus pre, minus post/minus pre (see table 3a). Mean values and standard deviations are displayed in table 3b.

Condition	*t*	*p*	*r*
Sham	3.6	0.0031	0.69
Minus	3.5	0.0039	0.68
Plus	2.9	0.012	0.61

Table 3a. Paired value t-test results comparing power at oIAF before and after stimulation for each condition.

Condition	Mean pre	Mean post	Std.Dev. pre	Std.Dev. post
Sham	1.70	1.41	0.62	0.44
Minus	1.71	1.47	0.60	0.66
Plus	1.67	1.40	0.56	0.47

Table 3b. Mean values and standard deviations for power at oIAF in pre and post stimulation recordings for each condition.

As can be seen in table 3a, all of these comparisons reached significance showing that there was a change in power at the oIAF for all stimulation conditions (both active and sham). Inspections of the mean values of the pre and post stimulation oIAF powers (table 3b) showed that in all three conditions the power was lower in the post-stimulation EEG recordings compared to the pre stimulation recordings.

A further analysis was then carried out to determine whether these changes occurred differentially for the active stimulation conditions compared to the sham stimulation condition. The power at the oIAF for the post stimulation recordings was subtracted from the oIAF power of the relative pre stimulation recording for each of the conditions. This resulted in a power difference value at the oIAF for each stimulation condition per participant. A repeated measures ANOVA (with the within subjects factor being stimulation condition; Sham, Plus, Minus) was then carried out on these difference values. No significant effect of stimulation condition was found ($F(2,28) = 0.092$; $p = 0.91$; $\eta_p^2 = 0.007$).

The t-test results and inspections of the mean values of each pre and post stimulation power value for each condition, show that the power at the original peak was reduced after the tACS or sham stimulation in all three stimulation conditions. However, this reduction did not occur to a different extent for the active conditions compared to the sham condition. Therefore these results suggest that the reason for the reductions in power is not a result of the tACS. Possible reasons could be that a general power decrease in alpha occurred after being at rest for 20 minutes in a dark room: this potential explanation is tested for below in question 4. An alternative explanation is that these findings could just show a regression to the mean as, by its very nature, an IAF peak would have a higher power than average for any given alpha frequency (see figure 5) and, as was apparent from the intra-individual variability in IAF across sessions found in this study, IAFs appear to be changeable across time (see also Vossen et al., 2015, for an account of IAF variability over time).

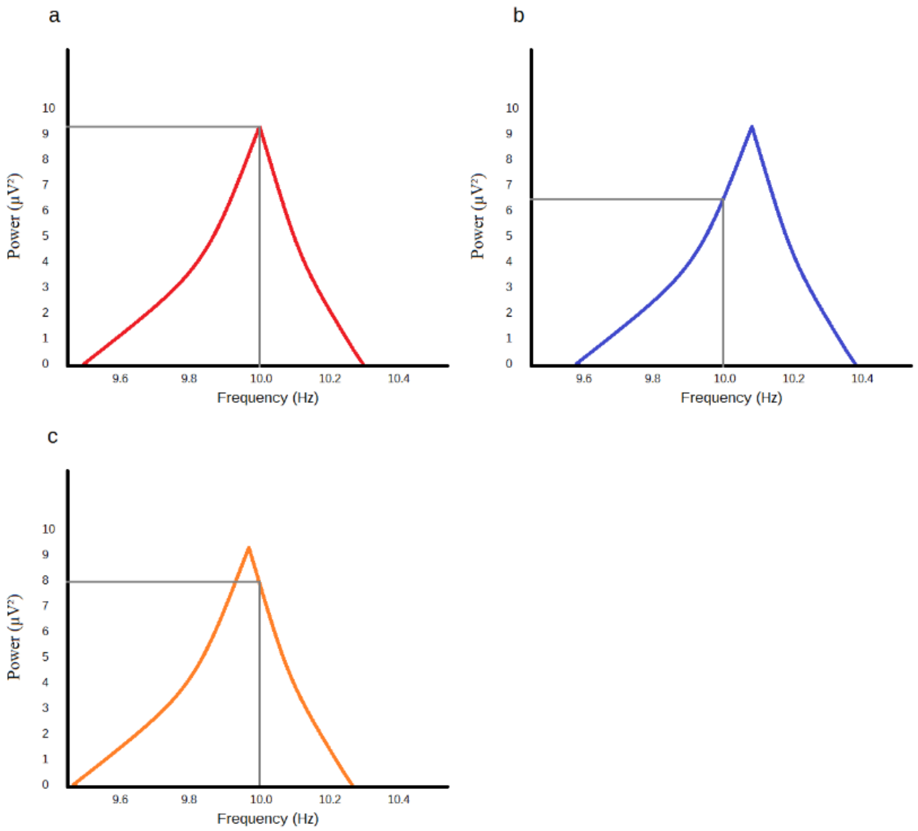

Figure 5. Three plots showing a natural alteration in IAF over time. Peak power has been kept constant for ease of demonstration. For this demonstration, the IAF at time point *a* will be considered the original IAF.

At time point *a* the IAF is 10Hz, by time point *b* it has moved to 10.1Hz, by time point *c* it has moved to 9.95Hz. The power at the original IAF (10Hz) has reduced at time points *b* and *c* as a natural consequence of 10Hz no longer being the peak.

4.3.4 Question 4: Did the overall power across the alpha band change after stimulation?

Average power values across the whole alpha band range (7-14Hz, samples taken at intervals of 0.125Hz) were calculated for each EEG recording. T-tests were conducted comparing the average powers for each pair of post and pre stimulation recordings for each stimulation condition (Sham Post/Sham Pre, Plus post/Plus pre, Minus Post/Minus pre). No significant difference in power was found for any of the conditions. See table 4.

Condition	*t*	*p*	*r*
Sham	0.98	0.34	0.25
Minus	0.41	0.69	0.11
Plus	-0.54	0.60	0.14

Table 4. Paired value t-test results comparing average alpha powers in pre and post stimulation recordings for each stimulation condition.

As no significant change in power occurred between the post and pre stimulation recordings, this rules out the general power decrease explanation of the results for analysis 3. Regression to the mean is therefore a more likely explanation.

To determine whether any significant differences in overall power occurred for the active conditions when compared to the sham stimulation condition, values for the difference in power between the post and pre stimulation recordings were calculated for each stimulation condition and a repeated measures ANOVA was conducted on the resulting values (within subject factor condition: sham, plus and minus). No significant effect of stimulation condition was found ($F(2,28) = 0.49$; $p = 0.62$; $\eta_p^2 = 0.034$) suggesting that no significant changes in overall alpha power occurred when sham was accounted for.

4.3.5 Summary of all analyses

In summary of all the analyses carried out, no significant differences in IAF or alpha power were found between the active tACS conditions compared to sham stimulation. A general decrease in power at the IAF occurred between the pre and post stimulation recordings, however this was not specific to the active stimulation conditions and was not part of a general shift in alpha power. Regression to the mean is a parsimonious explanation of this although no conclusive explanation can be given.

4.3.6 Variation of IAF across sessions

No in-depth analysis of individual data took place, however it was noted that the pre-stimulation IAF differed between sessions in all participants and therefore quick calculations were carried out to establish the range by which the IAFs varied.

The range of within participant differences in the pre-stimulation IAFs between sessions was 0Hz – 3.2Hz with an average range of 0.48Hz and an average standard deviation of 0.38Hz. One participant showed outlying differences in their range of IAFs therefore an outlier corrected range was also calculated excluding the data from this participant (range 0Hz - 1.1Hz, average range 0.36Hz, average standard deviation 0.16Hz).

Of the three pre-stimulation EEG recordings taken per participant, two were conducted on the same day (i.e. the recording before the sham session and the recording before the active session that formed the second half of the long session). Of these sessions, the range of differences in starting IAF was 0Hz – 1.1Hz (outlier corrected range = 0Hz – 0.5Hz) with an average range of 0.33Hz (outlier corrected = 0.27Hz) and a standard deviation of 0.23Hz (outlier corrected = 0.19Hz).

4.4 Discussion

In this study, off-peak alpha tACS did not produce any significant changes in the alpha activity of participants relative to sham stimulation that were detectable in subsequent offline EEG recordings. The main potential change of interest was a shift in the IAF. Such a shift was not found to have occurred. Additionally, three potential changes to alpha power were analysed – an overall shift in alpha power from active conditions relative to sham, a change in power around the frequency of stimulation and a change in power at the original (pre-stimulation) alpha peak. No changes in power across any of these areas were found when the active stimulation conditions were compared to sham stimulation. Therefore, in this study, off-peak right parietal alpha stimulation at 2Hz below and above IAF did not appear to successfully change cortical right parietal IAF or alpha power, relative to sham stimulation, in the minutes immediately following stimulation in a way that was apparent on EEG recordings. This next section will look into possible reasons why this may have occurred.

4.4.1 Possible reasons for no significant results

Entrainment may not have occurred

Firstly, it is possible that the off-peak stimulation did not cause measurable offline changes in frequency peaks or power because entrainment did not occur. One possible reason is that the particular parameters used in this study (electrode placement, stimulation frequencies, stimulation power) may not have been optimal. Previous studies suggest that off-peak entrainment is possible through tACS within the alpha band (e.g. Cecere et al., 2015; Minami & Amamo, 2017) and other frequency bands

(Vosskuhl et al., 2015), therefore, although the results from the current study were inconclusive, it is worth exploring the possibility of off-peak entrainment through tACS further to try to ascertain whether the specific parameters used in this study were not successful in producing entrainment and, if so, which parameter or combination of parameters were involved in this.

One possibility is that 2Hz may have been too far removed from the original IAF for successful entrainment to occur within this particular paradigm (i.e. resting state alpha). A systematic investigation of stimulation at varying frequencies both above and below the IAF would be a potentially useful future investigation to determine whether there are optimal stimulation frequencies for off-peak alpha entrainment. However, given the confounds of offline EEG recordings, such an investigation would be better suited to an MEG study or to the technique of combining online EEG recordings with an algorithm to reduce tACS artifacts (e.g. Helfrich et al., 2014). Both of these types of studies are beyond the scope of this PhD.

Online, but not offline, entrainment may have occurred

Another possibility is that the lack of offline entrainment is not indicative of a lack of online entrainment. Due to the technical issues of recording EEG during tACS (Feher & Morishima 2016; although see their study and Helfrich et al., 2014 for proposed solutions to these technical issues), the EEG recordings in this study were conducted straight after the stimulation had ceased. This study therefore investigated whether entrainment could occur that outlasted the period of stimulation (see Hanslymayr et al., 2014; Kasten et al., 2016 for evidence of after-effects from entrainment). If after-effects from entrainment had been found, it would have indirectly suggested that online entrainment was also occurring, however, the absence of any after-effects cannot be used as evidence that online entrainment did not occur.

Five minutes of EEG was recorded post-stimulation and averaged. Analysing a shorter segment of the post stimulation EEG recording may increase the chances of finding an entrainment echo if one is present (entrainment after effects have been found to last up to 70 minutes, however an effect known as the entrainment ‘echo' has been found to last approximately 1.5 seconds; Hanslmayr et al., 2014). However, analysing a shorter timeframe of EEG data would bring its own technical problems of increased noise and less reliable averages. Additionally, the post-stimulation EEG recordings were particularly noisy in terms of eye movements in the timeframe immediately following the stimulation (possibly due to generalised increased excitability; for example see Fresnoza et al., 2018, for an account of increased cortical excitability after tACS at 10Hz). This would further reduce the reliability of the averages taken from such a small time-frame.

4.4.2 Future directions

It is clear that the major limitation of this study was the lack of online EEG recordings. This meant there was an absence of measurement of whether physiological changes occurred whilst the tACS was being applied. This impedes any potential interpretation of non-significant results, as a lack of online changes cannot be extrapolated from a lack of offline changes in EEG. Whilst physiological changes (as opposed to cognitive or behavioural changes) were the focus of this study, it was decided that a way for further studies to circumnavigate this problem would be to use online measurements of behaviours that have a strong theoretical (and empirically supported) basis linking them to specific individual differences in frequencies. The theta-gamma theory of short-term/working memory proposed by Lisman and Idiart (1995) met these criteria particularly well, with strong predictions being able to be made regarding modifications in theta frequencies and (to a lesser extent) gamma frequencies and the subsequent effects these modulations may have on short-term/working memory performance. This theory will be discussed in detail in the next chapter. Then, in the chapters thereafter, there will be a report of three original studies, which use the theoretical implications of Lisman and Idiart's (1995) theory to explore the possibility of frequency modulation through off-peak theta and gamma entrainment.

Chapter 5: Models and neural correlates of working memory

5.1 Memory models/ Theories of memory

The idea that memory consisted of more than one separate store was first proposed by William James in the late 19th century (James, 1890). This theory suggested two stores: primary and secondary. These stores correspond to what is today referred to as short-term memory and long-term memory respectively (see the overview of the multi-store model of memory below for clarification of these terms). The idea of more than one separate memory stores has been expanded on since James' proposal in 1890 and more advanced multiple store models of memory have been proposed. Some of these models will now be outlined.

5.1.1 Multi-store model of memory

The multi-store model of memory was proposed by Atkinson and Shiffrin in 1968. This model states that memory is composed of three stores: the sensory register, short-term memory (STM) and long-term memory (LTM). Information that is processed by the senses enters the sensory register. If this information is attended to, it then enters STM. If information in STM is not attended to, through a process known as rehearsal (i.e. mental repetition of the information), then that information is lost from memory. However, through rehearsal, information can enter LTM from STM. In LTM information is stored for a long (potentially indefinite) duration. (Atkinson and Shiffrin, 1968).

According to this model, the sensory register has a different store for each sense, with these stores having a duration of up to half a second. STM has a duration of less than 30 seconds (Atkinson & Shiffrin, 1968) and has a capacity of approximately 7 items (Miller, 1955). According to Atkinson and Shiffrin (1968) auditory, visual and linguistic processing in STM are interlinked and therefore for the purpose of their model they considered STM to be a unitary store across these modalities. LTM is considered to have an indefinite time limit (Atkinson & Shiffrin, 1968; Craik & Lockhart, 1972).

Evidence for separate short-term and long-term memory stores

(i) Evidence from case studies of memory impairment

The separation of a short-term memory store and a long-term memory store has been backed up by evidence from patients with brain damage that affects one of these systems whilst leaving the other intact. For example, there have been case studies of LTM impairment with intact STM (Baddeley & Warrington, 1970; Scoville & Milner, 1957) and STM impairment with intact LTM (Shallice & Warrington, 1970); this lends support for the unidirectional aspect of this model, which states that information can only enter LTM through STM.

(ii) Evidence from the primacy and recency effects

Two well-known findings of empirical research that lend support to this model are the primacy and recency effects (Atkinson & Shiffrin, 1968; Glanzer & Cunitz, 1966; Page & Norris, 1998). When given a list of items to remember, people fairly consistently remember the earliest items on the list (the primacy effect) and the last items on the list (the recency effect) during subsequent recall tests. The explanation for this in line with the multi-store model is that the primacy effect is caused by the earliest items having been rehearsed and transferred to LTM during the memory task whereas items that were presented after the capacity of STM was reached were not able to be rehearsed and therefore were unable to enter LTM. The explanation for the recency effect is that later items from the list are remembered well as they are still in STM during the recall stage of the task, i.e. the latter items have not yet left STM even if they are not subsequently transferred to LTM through rehearsal (Atkinson & Shjiffrin, 1968; Glanzer & Cunitz, 1966). Further support for this explanation of the recency effect comes from a study by Postman and Phillips (1965) who delayed recall whilst giving participants a task that interfered with their ability to rehearse. This interference prevented the recency effect. This can be contrasted with a study by Baddeley and Hitch (1977) who also delayed recall but did not give participants a task to perform, thereby not interfering with the participants ability to rehearse any items in STM; In this study the recency effect was still present. However, see Bjork and Whitten (1974) for a criticism of primacy and recency effects.

Summary of the multi-store model

This model provided a well supported concept of separate stores for short-term and long-term memories. However, it has been criticised as being too simplistic to account for all aspects of encoding and recall (e.g. Baddeley & Hitch, 1974; Craik & Lockhart, 1972; also see Baddeley, 2010). Alternative theories of memory that were proposed in order to address these criticisms will now be looked at, starting with the levels of processing model proposed by Craik and Lockhart (1972).

5.1.2 Levels of processing model

Some of these criticisms were addressed by Craik and Lockhart in 1972 with their levels of processing model. This theory focused on the processes of memory rather than the structure of it and therefore cannot be considered to be either a multiple store model or unitary model of memory although it does distinguish between short-term and long-term retention of items in memory.

According to this theory there are three types of processing that can take place when encoding an item. Two of these fall under the category of shallow processing: Structural processing and phonemic processing. These focus on the visual aspects and auditory aspects of the item respectively. The third type of processing is semantic processing which is considered deep processing. This type of processing encodes using the meaning of the item. It involves top-down processing (where previous knowledge is drawn on to help give meaning to the item) and usually results in longer-term memory storage than the shallow processing methods (Craik & Lockhart, 1972).

This focus on memory processes, rather than memory structure, provided new understandings regarding how the type and depth of processing affects the likelihood of an item being successfully encoded into memory (Roediger et al., 2001). However, there were also many criticisms of this model (see Watkins, 2002 for an overview). Despite these criticisms, the ideas that this theory proposed concerning depth of processing and the role of active involvement during encoding have since been applied to structural models of memory. One such structural model of memory proposed by Baddeley and Hitch (1974) will now be outlined.

5.1.3 Working memory model

In 1974 Baddeley and Hitch proposed the working memory model as an alternative to the STM component of the multi-store model. They argued that the multi-store model's view of STM was too simplistic, particularly regarding its unidirectional and relatively passive nature. Baddeley and Hitch's new model reflected this with a more interactive alternative to STM, known as working memory (WM) which contained active processing and feedback loops. Similarly to the multi-store model, the working memory model considered a very temporary sensory memory to be the first step in the memory process, followed by short-term memory stores with items potentially being transferred to longer term storage. However, the nature of the short term memory storage in this model was very different to that proposed by Atkinson and Shiffrin and this alternative short-term store provides the crux of the model and will therefore be focused on here.

The originally proposed working memory model consisted of three components to the short-term memory storage, along with a sensory store and a long-term memory store. These three short-term components are the phonological loop, the visuospatial sketchpad and the central executive. The phonological loop and visuospatial sketchpads are short-term memory stores. The phonological loop stores auditory and language based items whilst the visuospatial sketchpad stores visual and spatial items in memory. There is empirical support for there being two distinct short-term stores for these types of items. For example, brain lesion case studies have demonstrated impairment in short-term memory across one modality but not another modality (Della Sala et al., 1999; Kasselimis et al., 2018). Additionally, empirical studies using interference tasks during a memory task have demonstrated that memory impairment is more pronounced when the interference task is of the same modality as the memory task (e.g. Della Sala et al., 1999).

The central executive acts as an attentional control system responsible for allocating memory resources between the phonological loop and visuospatial sketchpad. In this model, information can flow in both directions between the central executive and the two stores. Furthermore, information can flow in both directions between the central executive and the long-term memory store. This aspect is a departure from Atkinson and Shiffrin's (1968) multi-store model and shows memory to be a far more flexible and active process which includes aspects of top-down processing. Support for this aspect of the model comes from studies showing that prior knowledge plays a role in determining how likely a memory item is to be successfully encoded and recalled. For example, studies have shown that strings of letters are more successfully remembered when they consist of previously known acronyms compared to when they are random strings of letters of the same length. In these instances, the basic sensory information is comparable in each case (a string of letters); however, when information in long-term memory is taken into account, these groups of stimuli can no longer be considered comparable. Therefore, the findings that acronyms are more easily remembered suggests that information previously stored in LTM plays a role in the transfer of items from short-term stores to long-term stores, suggesting that the information flow between the two systems is bi-directional.

In 2000, Baddeley updated this model to include a new component, the episodic buffer (Baddeley, 2000). This component integrates information from the phonological loop, the visuospatial sketchpad and long-term memory to combine information stored in them into a cohesive whole.

5.2 Neural correlates of working memory

The remaining studies in this thesis utilise a working memory task to explore neural oscillations. Therefore, the neural corelates of working memory will now be explored.

Two main aspects can be considered to play a role in working memory performance: the absolute capacity of the memory store and the successful inhibition of irrelevant items (the latter being important due to the limited capacity of the WM store) (Sauseng et al., 2009) and two distinct neural processes have been linked to these two aspects of working memory performance. The role of inhibition in WM takes the form of preventing irrelevant items from being stored within WM and has been linked to alpha activity through both an increase in alpha power (Bonneford & Jensen, 2012; Sauseng et al., 2009) and a shift of alpha phase (Bonneford & Jensen, 2012). This is in line with other areas of cognition where alpha has been linked to inhibition of non-task related stimuli (e.g. Cooper et al., 2003; also see chapter 1). Absolute capacity in WM has been linked to theta activity. Power increases in theta around the parietal regions have been shown to be correlated to higher WM performance (e.g. Polania et al., 2012; Zakrzewska & Brzezicka, 2014). In addition to overall theta power playing a role in WM capacity, variations in WM load have been linked to variations in average theta frequency peaks. Axmacher et al., (2010) found that increasing task load in a WM task resulted in lower average theta frequencies. An increase in gamma power has also been linked to an increase in WM load (Howard et al., 2003). In addition to studies focusing separately on the roles of theta and gamma activity in WM capacity, studies have suggested that the interaction between theta and gamma oscillations during theta-gamma phase-coupling is a factor in WM capacity. For example, Kaminski et al., (2011) found evidence that the theta to gamma ratio plays a role in WM Capacity and Sauseng et al., (2009) found increased phase-synchronisation between gamma and theta oscillations with increased memory load during a WM task. One major theory for why this interplay between theta and gamma activity is important in WM capacity derives from work by Lisman and Idiart (1995). The implications from Lisman and Idiart's theory forms the theoretical basis of the remaining three studies in this thesis and will now be looked at in more depth.

5.2.1 Lisman and Idiart's theta-gamma theory of working memory capacity

This theory is extrapolated from a theory of short-term memory that was proposed by Lisman and Idiart (1995). It is worth noting that, whilst the original paper focused on short-term memory, this theory has been applied to both short-term memory (e.g. Kaminski et al., 2011; Sauseng et al., 2009) and working memory (e.g. Axmacher et al., 2010; Howard et al., 2003). In the three final studies of this thesis, this theory is applied to WM performance.

In their paper, Lisman and Idiart suggested that gamma cycles nested within theta cycles were responsible for storing items sequentially within short-term memory. It was proposed that each gamma cycle corresponds to one item stored in short-term memory and the theta cycles that they are nested within act as a binding mechanism, keeping the individual items together in one memory store. Each subsequent theta cycle acts as a boost, helping to maintain these items in the memory store. Lisman and Idiart (1995) used a computational model to support their theory, showing that it was theoretically possible for such a mechanism to exist. As further evidence, Lisman and Idiart noted that the average number of items that can be stored in short-term memory matches the average number of gamma cycles that can nest within one theta cycle. Since this model was proposed, empirical evidence has been provided for the theory (e.g. Kaminski et al., 2011; Sauseng et al., 2009).

5.2.2 Theta and gamma frequency peaks and individual differences in working memory capacity

The implications from Lisman and Idiart's theory regarding individual differences in theta and gamma peaks and their relationship to individual differences in WM capacity is seminal in the remaining three studies of this thesis. Therefore, this relationship will now be outlined.

To summarise Lisman and Idiart’s theory, it states that gamma cycles nested within theta cycles are responsible for the storage of items in working memory, and that each nested gamma cycle holds one memory item, with each theta cycle acting as a binding and boosting mechanism. If this is the case, it would follow that the number of gamma cycles that can potentially be nested within each theta cycle would correspond to the number of items that can potentially be stored in working memory. So, if the ratio between the dominant gamma and theta frequencies allowed a maximum of 6 gamma cycles to be nested within one theta cycle then it would follow that a maximum of 6 items could be stored in WM. In this respect, individual differences in the ratio of phase-coupled theta and gamma frequencies would reflect individual differences in WM capacity.

A person’s dominant gamma and theta frequencies could potentially influence their theta-gamma ratio, with individual differences in this ratio going on to reflect individual differences in WM capacity. In terms of theta activity, for the ratio to be maximised (i.e. with more gamma cycles being able to be nested within each theta cycle) the dominant theta frequency would need to fall towards the lower end of the theta frequency band. Conversely in terms of gamma activity, faster gamma activity would result in more gamma cycles being potentially nested within each theta cycle. So, if it is the case that theta and/or gamma peaks influence the ratio of phase-coupled theta-gamma, then lower theta frequency peaks and/or faster gamma frequency peaks would be expected to result in a higher working memory capacity.

To date, neurophysiological evidence has provided a correlational relationship between slower theta activity and greater WM capacity (Axmacher et al., 2010), however, Lisman and Idiart's theory suggests that the frequency of theta activity could play a *causal* role in WM capacity. If the length of each theta cycle or each gamma cycle were to change, the number of gamma cycles that could be nested within each theta cycle may be altered and WM capacity would subsequently change. The remaining studies in this thesis used the premise that theta and gamma frequency peaks can causally affect WM capacity as a way of behaviourally measuring frequency modulation through off-peak entrainment. Studies 2 and 3 investigated the effects of off-peak theta tACS on a WM task to see if any subsequent changes in WM performance occurred, which would suggest successful frequency modulation within the theta band. Study 4 applied off-peak gamma tACS to the same behavioural paradigm to test for frequency modulation within the gamma band. These studies will be described in the following chapters.

[The study in this chapter along with the study in chapter 7 have been published together as a research article, please see appendix 1 for this article.]

Chapter 6: Study 2:

The modulation of working memory capacity through off-peak theta stimulation

6.1 Introduction

6.1.1 The link between ITF and WM capacity

As mentioned in the previous chapter, there is both theoretical and empirical support that individual differences in theta frequency peaks (individual theta frequency – ITF) could be related to individual differences in working memory (WM) capacity (Axmacher et al., 2010; Lisman & Idiart, 1995). Specifically, lower ITF is thought to be related to a higher WM capacity and higher ITF to a lower WM capacity. Extrapolations from Lisman and Idiart's theta-gamma theory of working memory capacity (1995) suggest that there is a strong possibility that this relationship is causal, with the ITF directly causing changes in WM capacity (see previous chapter for a more detailed overview of this including a description of Lisman and Idiart's theory).

This study utilised the strong predictions that could be made regarding individual theta frequencies and WM performance from Lisman and Idiart's (1995) theta-gamma theory of working memory capacity. These predictions, along with empirical support from correlative studies (e.g. Axmacher et al, 2010; Sauseng et al. 2009, please note, this latter study referred to short term memory, see previous chapter for more details on both of these studies) allows WM performance to be used as an indirect behavioural measure of ITF modulation in an off-peak theta stimulation study. Whilst such behavioural measures can only be at most indicative of physiological changes to theta activity, such measures can be taken during stimulation and thus are not susceptible to the limitations of offline EEG recordings that were present in study 1.

6.1.2 Aims of this study

Therefore, this study had two main aims; firstly it further investigated the theta-gamma theory of WM capacity in regards to the causal relationship between ITF and WM capacity and, secondly, it provided an opportunity to investigate the online effects of off-peak tACS through utilising a cognitive function linked to differences in frequency peaks that had strong theoretical support. Whilst a behavioural study obviously has limitations for physiological interpretation, the previous neurophysiological correlational support of this theory alongside its strong theoretical basis of causality provided a way of investigating online effects of tACS whilst circumventing the technical issues surrounding concurrent EEG recordings outlined in study 1 (see also chapter 2, section 2.8, and chapter 3, section 3.5). Whilst definitive neurophysiological evidence cannot be interpreted from a behavioural study, the strongly supported and bidirectional behavioural predictions of this theory would allow for the possibility of a tentative inference to still be suggested in the event of the predicted results being found.

If theta frequency does play a causal role in WM capacity, rather than merely being a correlated factor, it would be expected that successful entrainment at low and high theta frequencies would result in an increase and decrease in WM capacity respectively. Therefore, if WM capacity is altered in the expected directions as a result of the high and low theta stimulation, this would not only provide support that entrainment at off-peak frequencies can occur within the theta frequency band but would also provide evidence to support the idea that the dominant frequency of theta activity is a causal factor in WM capacity. Additionally, this would provide evidence that off-peak entrainment can be used successfully to alter cognitive performance.

As mentioned in chapter 1, the theta frequency range spans 4Hz-7Hz. Therefore, in this study, 4Hz was used as the low theta frequency and 7Hz as the high theta frequency. It was expected that stimulation at 4Hz would improve performance in a WM capacity task and stimulation at 7Hz would impair performance in the same task. Stimulation was focused on the parietal region as this area has been linked to WM (e.g. McCollough et al., 2007; Vogel et al., 2005; Vogel & Machizawa, 2004) and its relationship with theta activity (e.g. Sauseng et al., 2009). Stimulation was unilateral (in this case focused on the right hemisphere) and an additional prediction was made that any results found would be confined to the visual hemifield contralateral to the site of stimulation (i.e., in this experiment, the left visual field).

6.2 Method

6.2.1 Participants

16 participants (9 female) with a mean age of 22.8 years (± 5.2) (range 18-37) took part in the experiment. Participants gave written consent to take part and completed a tACS safety screening questionnaire before each session. The study was approved by the local ethics committee.

6.2.2 Design

A repeated measures design was used with the within subjects factors being stimulation condition, hemifield and memory load. There were two active stimulation conditions (4Hz and 7Hz tACS) and one control condition (sham stimulation). These stimulation conditions took place during three separate sessions that occurred on separate days with the order counterbalanced across participants. At least 24 hours passed between different sessions. A partial-double blind design was used. The participants were unaware that different stimulation protocols were being used for each session and were unaware that one session consisted of sham stimulation. Due to limitations of the stimulation device, the experimenter was aware of whether a particular session consisted of sham or active stimulation however they were unaware of whether each active stimulation session consisted of stimulation at 4Hz or 7Hz.

6.2.3 Stimulation protocol

Two 5x7cm electrodes were placed over the scalp using saline soaked sponges (100mM solution). 5x7cm electrodes were chosen in order to provide general, non-focalised coverage of the parietal region of the frontoparietal theta pathway. Prior to electrode placement skin was prepped with alcohol wipes to improve conductivity. One electrode was placed over the right parietal cortex (corresponding to P4 according to the 10-20 system, see Klem et al., 1999) and the other was placed on the vertex (corresponding to Cz according to the 10-20 system). Stimulation was delivered using a Magstim DC-Stimulator Plus device. Stimulation was delivered at 1500μA (chosen as a standard stimulation intensity, e.g. Feurra et al., 2011; Laczo et al., 2012; Neubauer et al., 2017, which was likely to be effective yet unlikely to cause visual disturbances). None of the participants reported visual disturbances at this level during any session.

All conditions consisted of a ramping up stage at the beginning of the stimulation. For the 4Hz condition this lasted for 120 cycles, for the 7Hz condition this lasted for 210 cycles and for the sham condition this lasted for 165 cycles. For the 4Hz and 7Hz conditions this was followed by stimulation at 1500μA for the remainder of the behavioural task. For the sham condition the ramping up period was followed by stimulation for 165 cycles and then a cooling down period that lasted 165 cycles. The brief stimulation period in the sham condition was carried out to mimic any sensations that may have been felt during the active stimulation conditions. During the ramping up, stimulation and cooling down periods in the sham condition, the frequency of stimulation was set to 5.5Hz. This frequency was chosen as a neutral frequency relative to the two active conditions. Throughout all stimulation sessions, impedance levels were kept below 5kΩ.

6.2.4 Materials

A variation of the visual delayed match to sample task based on Vogel and Machizawa (2004) was used. Two arrays of coloured squares were situated on either side of a white fixation cross in the centre of a black screen. The number of squares in each array (the memory load) was 4, 5 or 6. The left and right arrays were always different from each other in any given trial; however, the number of squares in each trial was always the same for the left and right arrays. See figure 6b.

6.2.5 Procedure

Behavioural task

The task started with a fixation cross on the screen. Prior to presentation of the arrays, an arrow briefly appeared on the screen (200ms) to indicate which of the two upcoming arrays (left or right) needed to be memorised. The two arrays (one on the left and one on the right of the fixation cross) then appeared on the screen for 100ms. This was followed by a retention interval of 900ms during which there was a fixation cross on the screen. After the retention interval, two arrays appeared on the screen for 2000ms (again, there was one array either side of a fixation cross). See figure 6a. Participants had to indicate whether this array on the previously indicated side was the same or different to the array on that side presented previously during that trial. This was done by pressing either the left or right button of the mouse (left to indicate that the arrays were the same, right to indicate that they were different). The order of trials within each block was randomised by the computer program.

There were 6 blocks of 20 trials presented during each stimulation session. Each block contained trials of one load only (4, 5 or 6). For each session, the blocks were chosen at random from a set of 9 blocks (3 for each load) with the following restrictions: exactly 2 blocks of each load were to be used and no block was to be repeated during any given session. These 6 blocks were presented in a randomised order. Each block contained 5 left mismatched, 5 left matched, 5 right mismatched and 5 right matched trials.

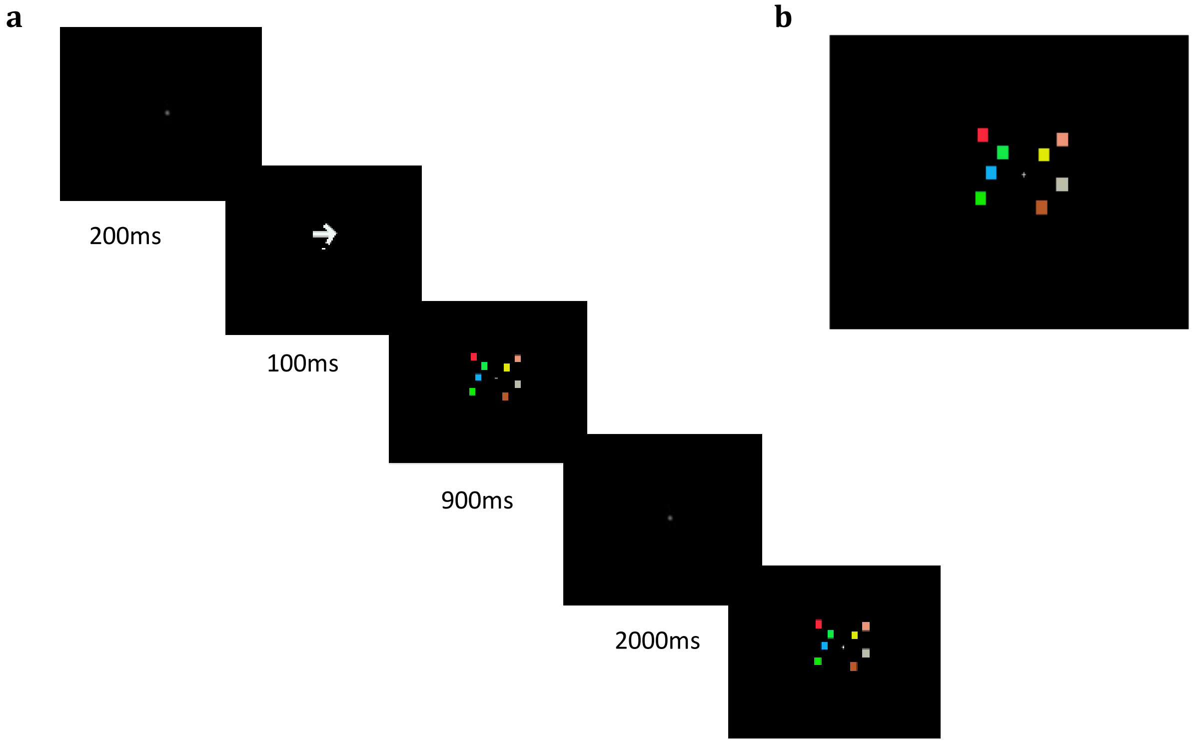

Figure 6. (a) Behavioural task. (b) Example of stimulus from a memory load 4 trial.

Experiment procedure

After the set-up of the electrodes, participants were presented with written instructions. Participants were instructed to use only one hand to make their responses and were given the opportunity to clarify the instructions if needed. The lights were turned off and participants carried out a practice block of trials. This practice block used load 3 stimuli to prevent load-specific practice effects affecting subsequent performance.

After the practice block had finished, additional saline solution was added to the sponges and stimulation was started. Participants were then instructed to start the first block when ready. After each experimental block, participants pressed the enter button to continue to the next block. Each block lasted approximately 2 minutes therefore the total task time during stimulation lasted approximately 12 minutes. Stimulation continued throughout the duration of the task. Participants were given monetary compensation or course credits for their participation after each of the three sessions and were debriefed after the final session and invited to ask any questions.

6.3 Results

6.3.1 Pre-processing of data into k values

The raw behavioural data was processed into k values (a standardised measure of WM capacity; see e.g. McCollough et al., 2007; Sauseng et al., 2009; Vogel et al., 2005; Vogel & Machizawa, 2004) for each combination of load x hemisphere x session per participant.

This resulted in a total of 18 k values per participant (6 values per stimulation session). These values were calculated with the formula: k = (hit rate – false alarms) * set size/10.

6.3.2 Repeated measure ANOVA

A repeated measure ANOVA was conducted with the within subjects factors stimulation (4Hz, Sham and 7Hz), load (4 items, 5 items and 6 items) and hemifield (left and right). Means and standard deviations were also calculated.

A significant main effect of load was found ($F(2,30) = 9.39$, $p = .0007$, $\eta_p^2 = 0.39$). Inspection of the means showed that, as expected, the higher the load, the lower the k value (i.e. accuracy decreased with an increase in memory load) (mean k values; load 4 = 2.14 (sd. 0.85), load 5 = 1.71 (sd. 0.86), load 6 = 1.42 (sd. 0.87)). No other main effects reached significance. No interactions reached significance. Mean k values for stimulation condition were as follows: 4Hz = 1.86 (sd. 0.75), sham = 1.65 (sd. 0.90), 7Hz = 1.76 (sd. 0.92).

The results suggest that the tACS did not significantly alter WM performance.

6.4 Discussion

6.4.1 Summary of the results

The results from this study suggest that the stimulation was not successful in altering WM performance. Possible reasons for this will now be discussed. It is possible that off-peak theta stimulation did not cause modulation of theta frequencies in this instance. Alternatively, it may be the case that modulation of theta frequencies did occur, yet changes in ITF do not causally change WM performance. Another possibility is that modulation of theta frequencies did occur and changes in ITF can causally change WM performance, however, in this particular instance, such changes did not translate into changes in WM performance.

As no physiological recordings were taken, it cannot be inferred from this study whether frequency modulation did or did not occur and it can also not be deduced whether or not such modulation is possible.

Further studies could help to shed light on why no significant changes in WM performance were found. Ideally, physiological recordings would be taken during stimulation, however, a behavioural task was chosen for the very purpose of circumnavigating the technical issues presented by online physiological recordings. In light of the non-significant results, one potential issue with the parameters of this study was identified as a possible confound and this shall now be discussed in the following section.

6.4.2 Potential desynchronization across the frontoparietal theta network

In this study the electrode montage of P4 and Cz was used. P4 was chosen as it lies over the frontoparietal theta network, a cortical pathway that has been linked to WM performance (e.g. Polani et al., 2012). Additionally, P4 was chosen as it lies over the right hemisphere and theta-gamma phase coupling has been shown to be stronger in the right hemisphere (comparative to the left hemisphere) during matched items in a working memory task (Holz et al., 2010). Cz was chosen as it is a commonly used position for the second electrode in tACS studies. However, Cz lies on the perimeter of the frontoparietal theta pathway and additionally, it spans the network across both hemispheres. Therefore, in addition to the pathway stimulation occurring under the electrode at P4, the electrode at Cz could have stimulated a more anterior part of the frontoparietal theta pathway and could have stimulated it across both hemispheres. The potential problem with this will be outlined below.

With tACS entrainment studies, each electrode essentially plays the role of an external oscillator. In theory, these oscillators would work independently from each other with regards to the entrainment of brain oscillations. With alternating current stimulation, the current beneath each electrode would be 180° out of phase with the current beneath the other electrode. Therefore, if both electrodes spanned one neural network this could cause desynchronisation across this network. As both electrodes in this montage spanned the network of interest (the frontoparietal theta pathway) this montage could have potentially caused such desynchronisation.

In terms of cognitive functioning, the impact of synchronisation and desynchronisation of neurons is not only seen at a localised level. It has been shown that the synchronisation and desynchronisation of neurons across spatially separated brain areas can also have an impact on cognitive functions (e.g. Varela et al., 2001). In a study specifically investigating theta activity and WM, Jones and Wilson (2005) demonstrated that theta desynchronisation between specific regions of the hippocampus and prefrontal cortex can impair WM capacity. Whilst the specific brain areas focused on in Jones and Wilson's study differed from the current study and additionally it focused on WM in rats, it provides evidence that desynchronisation across two areas of a WM related theta network can have an impact on WM performance.

Therefore, it is possible that desynchronisation across the frontoparietal theta network produced unanticipated behavioural effects in the current study. It is not clear how any such effects may have interacted with any potential frequency modulation effects and whether any such interactions would have differed between the two active stimulation conditions, therefore the possibility of this network desynchronisation can be viewed as a potential confound.

To address this potential confound, a further experiment, study 3, was devised which repeated the general aims of this study but eliminated this potential desynchronisation through the use of an alternative electrode montage. Study 3 will now be outlined in the following chapter and the results from study 2 will be discussed further in relation to the findings from study 3.

Chapter 7: Study 3:

The effect of electrode montage in the modulation of working memory capacity through off-peak theta stimulation

7.1 Introduction

7.1.2 Aims of the current study

Study 3 aimed to test the hypothesis that the montage used in study 2 caused desynchronisation across the frontoparietal theta network which resulted in a lack of significant changes to WM performance. For this study, an alternative montage was used where only the electrode placed at location P4 lay across this network. The other electrode was placed on the forehead above the right eyebrow (right supraorbital). All other variables were kept the same as in study 2 (including the position of the electrode at P4) in order to allow for a direct comparison to be made between the results from the two electrode montages.

7.2 Method

7.2.1 Power analysis

Based on a power analysis conducted on Vosskuhl et al., (2015), actual effect size f = 0.47, with a conservative estimated effect size of f= 0.25, alpha = 0.05 and 80% power, a total sample size of 28 participants (14 per group) is suggested. In study 2, 16 participants were used and 16 were again used in study 3, making a total cross-montage comparison sample size of 32.

7.2.2 Participants

16 new participants (7 female) took part in the study. The mean age was 28.3 years (± 7.6) (range 21-51 years). Participants gave written consent to take part and completed a tACS safety screening questionnaire before each session. The study was approved by the local ethics committee.

7.2.3 Design

As in study 2, a repeated measures design was used with the within subjects factors being stimulation condition, hemifield and memory load. As with study 2, there were two active stimulation conditions (4Hz and 7Hz tACS) and one control condition (sham stimulation) and these stimulation conditions took place during three separate sessions that occurred on separate days with the order counterbalanced across participants. At least 24 hours passed between different sessions. For this montage, a single blind design was used, with the participants unaware that different stimulation protocols were being used for each session and unaware that one session consisted of sham stimulation. As one of the electrodes was positioned close to the right eye, there was a high likelihood of visual disturbances (phosphenes and flicker) occurring with this electrode montage and in many cases it was necessary to adjust the power of stimulation to suit each participant. Due to limitations of the stimulation device this power adjustment meant that the experimenter was aware of which stimulation condition was being carried out during each session. This single blind format was in contrast to the partial-double blind format of study 2 (see section 6.2.2). Due to the different electrode positions in study 2, no visual disturbances were reported. This meant that no power adjustments needed to be made and this allowed for a partial double-blind format to be used in study 2 which was not possible during study 3. However, given the nature of the study, and in particular the lateralised nature of the predicted results, a single blind design was considered adequate.

7.2.4 Stimulation protocol

Two 5x7cm electrodes were placed over the scalp using saline soaked sponges (100mM solution). Prior to electrode placement skin was prepped with alcohol wipes to improve conductivity. One electrode was placed over the right parietal cortex (corresponding to P4 according to the 10-20 system; see Klem et al., 1999) and the other was placed on the forehead above the right eyebrow (supraorbital). Stimulation was delivered using a Magstim DC-Stimulator Plus device.

During the first session, stimulation at 1500μA was tested to see if retinal phosphenes or flicker occurred. If none occurred, this level of stimulation was used. If retinal phosphenes or flicker occurred at this level, the individual threshold for these visual disturbances was determined for that participant. This was done by starting at 1000μA and increasing in increments of 25μA or decreasing in increments of 100μA and then back up in increments of 25μA until the highest level that did not produce retinal phosphenes was found. The average level of stimulation was 1238μA (±298μA), with 8 participants requiring a reduction in intensity. The average reduction of intensity (across sessions where a reduction occurred) was 554μA (range: 250-850μA). For the second and third sessions the voltage determined in the first session was used. In three cases, visual disturbances were reported at this level during the second or third session and the voltage was therefore reduced for the remaining sessions for those three participants (using the method outlined above to determine the appropriate level). In these cases this resulted in differences of -50μA, -650μA and -400μA for the 7Hz session relative to the 4Hz session. No other participants reported retinal phosphenes at the threshold determined during the first session.

As with study 2, all conditions consisted of a ramping up stage at the beginning of the stimulation. For the 4Hz condition this lasted for 120 cycles, for the 7Hz condition this lasted for 210 cycles and for the sham condition this lasted for 165 cycles. For the 4Hz and 7Hz conditions this was followed by stimulation at the predetermined threshold or at 1500μA, for the sham condition the ramping up period was followed by stimulation for 165 cycles and then a cooling down period that lasted 165 cycles. The brief stimulation period in the sham condition was carried out to mimic any sensations that may have been felt during the active stimulation conditions. This also allowed the visual disturbance threshold to be determined when the first session contained sham stimulation. During the ramping up, stimulation and cooling down periods in the sham condition, the frequency of stimulation was at 5.5Hz. This frequency was chosen as a neutral frequency relative to the two active conditions. Throughout all stimulation sessions, impedance levels were kept below 5kΩ.

7.2.5 - 7.2.6 Materials and procedure

The materials, behavioural task and experiment procedure were the same as in study 2 and will be outlined below.

7.2.5 Materials

A variation of the visual delayed match to sample task based on Vogel and Machizawa (2004) was used. Two arrays of coloured squares were situated on either side of a white fixation cross in the centre of a black screen. The number of squares in each array (the memory load) was 4, 5 or 6. The left and right arrays were always different from each other in any given trial; however, the number of squares in each trial was always the same for the left and right arrays. See figure 7b.

7.2.6 Procedure

Behavioural task

The task started with a fixation cross on the screen. Prior to presentation of the arrays, an arrow briefly appeared on the screen (200ms) to indicate which of the two upcoming arrays (left or right) needed to be memorised. The two arrays (one on the left and one on the right of the fixation cross) then appeared on the screen for 100ms. This was followed by a retention interval of 900ms during which there was a fixation cross on the screen. After the retention interval, two arrays appeared on the screen for 2000ms (again, there was one array either side of a fixation cross). See figure 7a. Participants had to indicate whether this array on the previously indicated side was the same or different to the array on that side presented previously during that trial. This was done by pressing either the left or right button of the mouse (left to indicate that the arrays were the same, right to indicate that they were different). The order of trials within each block was randomised by the computer program.

There were 6 blocks of 20 trials presented during each stimulation session. Each block contained trials of one load only (4, 5 or 6). For each session, the blocks were chosen at random from a set of 9 blocks (3 for each load) with the following restrictions: exactly 2 blocks of each load were to be used and no block was to be repeated during any given session. These 6 blocks were presented in a randomised order. Each block contained 5 left mismatched, 5 left matched, 5 right mismatched and 5 right matched trials.

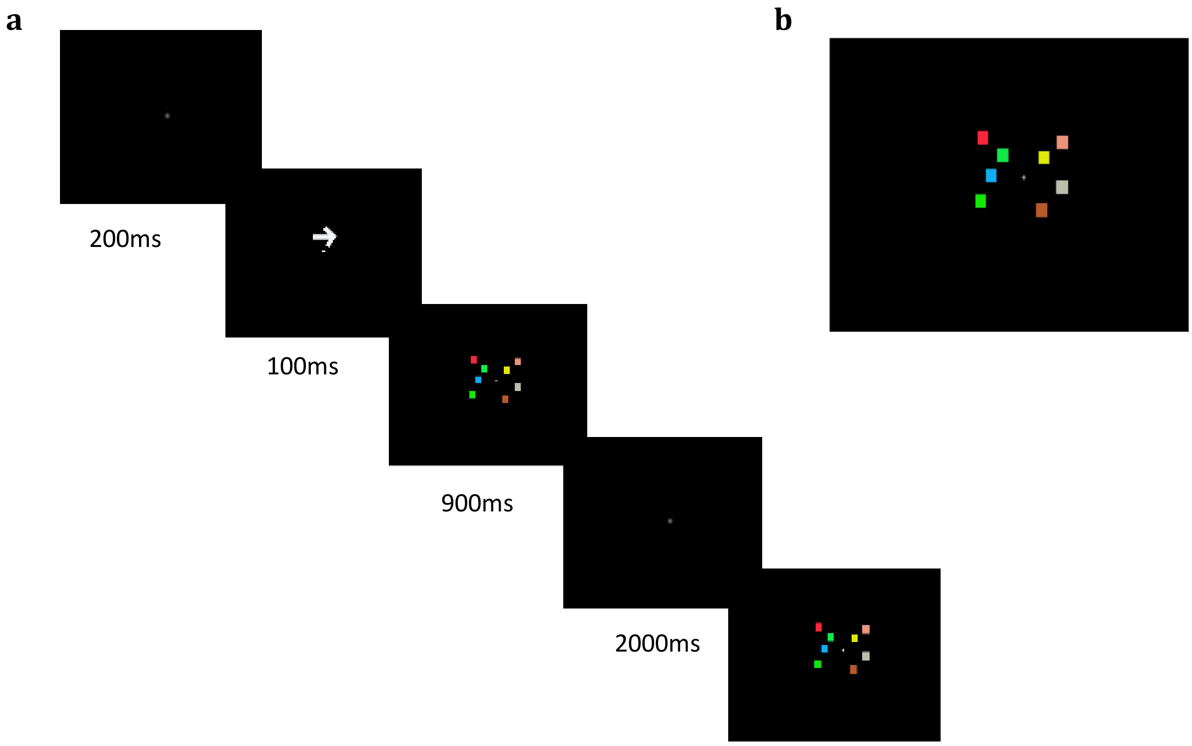

Figure 7. (a) Behavioural task. (b) Example of stimulus from a memory load 4 trial.

Experiment procedure

After the set-up of the electrodes, participants were presented with written instructions. Participants were instructed to use only one hand to make their responses and were given the opportunity to clarify the instructions if needed. The lights were turned off and participants carried out a practice block of trials. This practice block used load 3 stimuli to prevent load-specific practice effects affecting subsequent performance.

After the practice block had finished, additional saline solution was added to the sponges and stimulation was started. Participants were then instructed to start the first block when ready. After each experimental block, participants pressed the enter button to continue to the next block. Each block lasted approximately 2 minutes. Therefore the total task time during stimulation lasted approximately 12 minutes. Participants were given monetary compensation or course credits for their participation after each of the three sessions and were debriefed after the final session and invited to ask any questions.

7.3 Results

7.3.1 Pre-processing of data into k values

The raw behavioural data was processed into k values (a standardised measure of WM capacity; see e.g. McCollough et al., 2007; Sauseng et al., 2009; Vogel et al., 2005; Vogel & Machizawa, 2004) for each combination of load x hemisphere x session per participant.

This resulted in a total of 18 k values per participant (6 values per stimulation session). These values were calculated with the formula: k = (hit rate – false alarms) * set size/10.

7.3.2 Repeated measure ANOVA

A repeated measure ANOVA was conducted with the within subjects factors stimulation (4Hz, Sham and 7Hz), load (4 items, 5 items and 6 items) and hemifield (left and right). Means and standard deviations were also calculated. The main effect of stimulation approached significance ($F(2,30)= 3.29$, $p=.05$, $\eta_p^2 = 0.18$) and there was a significant main effect of load ($F(2,30)= 19.12$, $p < .00001$, $\eta_p^2 = 0.57$). Inspection of the means for load showed that, as expected, the higher the load, the lower the k value (mean k values: load 4 = 2.00 (sd. 0.79), load 5 = 1.54 (sd. 0.70), load 6 = 1.14 (sd. 0.84)). Analysis of the sham only trials also showed a significant main effect of load ($F(1,30) = 9.51$, $p=.001$, $\eta_p^2 = 0.39$) (mean k values: sham load 4 = 2.10 (sd. 0.78), sham load 5 = 1.53 (sd. 0.71), sham load 6 = 1.13 (sd. 0.92)). Paired t-tests were carried out on all paired combinations of load. As can be seen from table 5a, all differences reached significance. Paired t-tests were also carried out on all paired combinations of load for the sham condition only. As can be seen from table 5b, there were significant differences between load 4 and 5 and load 4 and 6 but the difference between load 5 and 6 did not reach significance.

	t	*p*	*r*
Load 4/Load 5	3.66	0.002	0.69
Load 5/Load 6	2.76	0.015	0.58
Load 4/Load 6	6.11	<0.0001	0.85

Table 5a. Paired t-tests for load conditions

	t	*p*	*r*
Sham Load 4/Sham Load 5	3.55	0.003	0.68
Sham Load 5/Sham Load 6	1.715	0.107	0.41
Sham Load 4/Sham Load 6	3.70	0.002	0.69

Table 5b. Paired t-tests for load conditions for sham only trials

Inspection of the means for stimulation showed that, as predicted, stimulation at 4Hz produced the highest k values (mean k = 1.78 (sd. 0.77)), stimulation at 7Hz produced the lowest k values (mean k = 1.33 (sd. 1.04)) and sham stimulation produced k values that fell between the k values for the two active stimulation conditions (mean k = 1.59 (sd. 0.62)). A significant interaction was found between stimulation condition and hemifield ($F(2,30) = 3.61$, $p = .037$, $\eta_p^2 = 0.17$). No other main effects or interactions reached significance.

Given the significant interaction between stimulation condition and hemifield, subsequent analyses were conducted to explore the effects of hemifield further.

The results were separated into two datasets, one for right hemifield and one for left hemifield and a repeated measures ANOVA was conducted on each dataset with the within subjects factors stimulation condition and load.

For left hemifield trials there were significant main effects of stimulation condition (*F*(2,30) = 8.57, *p*=.001, η_p^2 = 0.37) and load (*F*(2,30) = 5.49, *p*=.01, η_p^2 = 0.26). Again, the directions of all mean k values were as predicted (mean k for 4Hz = 1.98 (sd. 0.78), mean k for sham = 1.62 (sd. 0.91), mean k for 7Hz = 1.20 (sd. 0.92)). There were no significant interaction effects.

For the right hemifield trials there was a significant effect of load (*F*(2,30) = 12.41, *p*=.0001, η_p^2 = 0.45). No other main effects or interactions reached significance.

The results from these two further ANOVAs show that the effects of the tACS occurred on the hemifield contralateral to the stimulation but not on the hemifield ipsilateral to stimulation.

7.3.3 Comparison of results from study 2 and study 3

The k values from studies 2 and 3 were compared in a mixed ANOVA, with the between subjects factor being montage (Cz and supraorbital) and the within subjects factors being condition (4Hz, sham, 7Hz), load (4, 5, 6) and hemifield (left, right). Results showed a main effect of load (*F*(2,60) = 26.40, *p*<.00001, η_p^2 = 0.47). There was a marginal interaction between condition x hemifield x montage (*F*(2,60) = 2.72, *p*= .074, η_p^2 = 0.083), suggesting that the two montages had a different impact on the efficacy of the stimulation.

7.4 Discussion

The findings of study 3 suggest that tACS delivered at low and high theta frequencies can improve and worsen visuospatial WM performance respectively (specifically, the maintenance of items in working memory; see Sauseng et al., 2009). These findings support the idea that frequency peaks can be successfully modulated through off-peak entrainment and that such modulation can produce tangible behavioural effects. The findings also lend support to Lisman and Idiart's theta-gamma theory of working memory (Lisman & Idiart, 1995) and provide evidence that theta frequency plays a causal role in determining WM capacity, rather than just being a correlational factor. The lateralisation of the results from this study also suggest that there is a spatially organised element to the frontoparietal theta pathway in regards to visuospatial WM with visuospatial WM items being processed in the contralateral hemisphere. The implications of these results for off-peak entrainment and for the theta-gamma WM theory (Lisman & Idiart, 1995) will now be discussed in turn.

7.4.1 The results in the context of off-peak entrainment.

As with any behavioural study, this study has limitations regarding how much it can reveal about neural oscillations and any suggestions as to what it reveals on a physiological level need to be treated with caution. However, in this particular case, tentative inferences regarding the behaviour of theta activity appear to be justified. Previous studies have provided physiological support of a correlative nature for the theta-gamma theory of WM capacity (e.g. Kaminski et al., 2011; Sauseng et al., 2009).

Furthermore, the theta-gamma theory provides strong bidirectional behavioural predictions that would follow from any potential causal relationship between theta frequency and WM capacity. This study was able to test these predictions and found the results to support them in both directions, with a control condition indicating that both high and low theta stimulation resulted in the expected results relative to no stimulation. Therefore, this behavioural study supports a tentative inference that the behavioural results found were a result of off-peak theta stimulation altering the theta frequency peaks in the direction of stimulation. Specifically, stimulation at 4Hz slowed down theta activity and stimulation at 7Hz sped up theta activity by increasing power at the frequency of stimulation through the process of off-peak entrainment (see section 3.3.2 for an explanation of this process). Whilst this study provides no physiological evidence to directly support this, it is a likely, theory-based, explanation for the behavioural results.

7.4.2 The results in the context of the theta gamma theory of WM capacity

This study provides the first behavioural evidence that changes in the speed of theta oscillations can cause both an increase and a decrease in WM capacity. Specifically, it provides evidence that slower theta frequencies cause an increase in WM capacity and faster theta frequencies cause a decrease in it. As well as providing further support in general for the theta-gamma theory of WM capacity (Lisman & Idiart, 1995), this study provides support for one of the extrapolations that can be made from the original theory by providing evidence that differences in theta peaks are causally responsible for differences in WM capacity. This supports the suggestion that individual differences in theta frequency can have a causal effect on individual differences in WM capacity.

7.4.3 Other possible explanations for the results

It was mentioned in chapter 5 that WM performance is determined by a combination of both absolute capacity and the successful inhibition of the storing of irrelevant items. One alternative explanation of the results is that stimulation at 4Hz increases the inhibition of irrelevant items on the side ipsilateral to the stimulated hemisphere and stimulation at 7Hz decreases this inhibition. As 7Hz could be considered to fall towards the lower end of the alpha range it could be possible that such stimulation affected alpha activity in a way that disrupted this inhibition process (an increase in alpha desynchronisation or a lowering of IAF towards 7Hz would be the two most likely causes). However, this explanation cannot account for the finding that stimulation at 4Hz increased WM performance. It is unlikely that one mechanism is responsible for the results of 7Hz stimulation and another mechanism responsible for the results of 4Hz stimulation, particularly given the strong lateralised effect seen in both conditions and the fact that the results from the sham stimulation condition fell between the two active conditions. Furthermore, oscillations at 7Hz over the parietal regions are more linked to WM theta activity than inhibitory alpha activity. However, whilst a reduction of IAF is an unlikely explanation as it cannot account for the results found with stimulation at 4Hz, it is possible that theta stimulation over the prefrontal cortex is responsible for the alteration of WM found in study 3, as prefrontal stimulation did not occur in study 2. This could potentially explain the discrepancy between the results of study 2 and study 3, as an alternative to the previously discussed theory that stimulation at Cz caused desynchronization across the frontoparietal theta network

7.4.4 Comparison of the two montages using a current flow analysis

To gain more insight into the different effects of the montages on stimulation area, current flow strengths were calculated for both montages (based on a realistic head model, Miranda et al. 2013). The results are shown in figure 8.

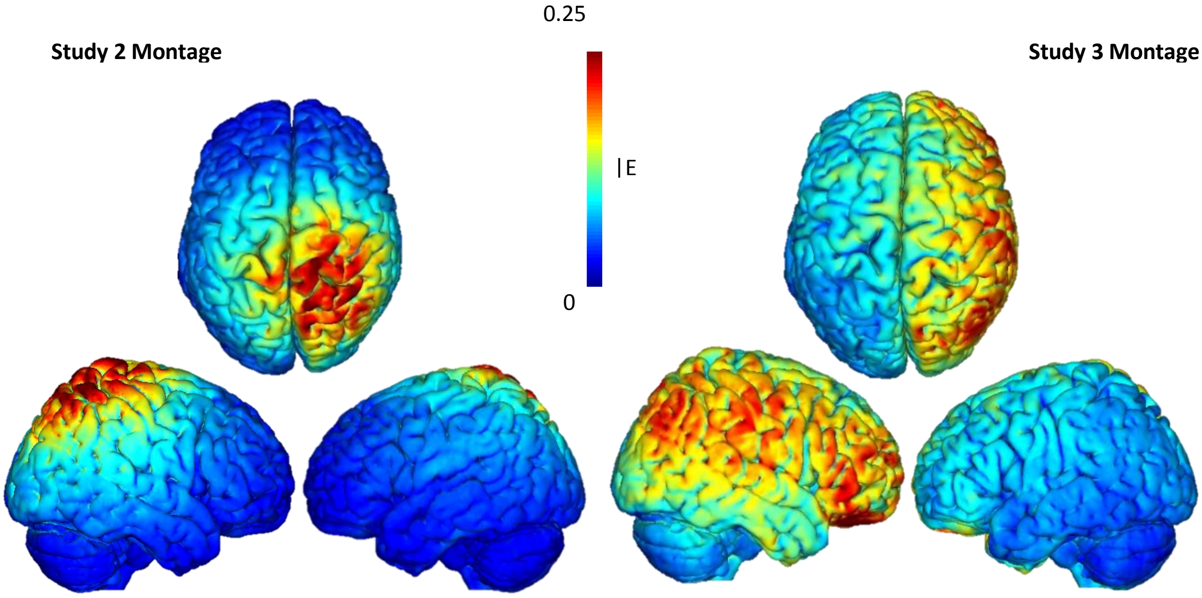

Figure 8. Current flow analysis comparing the electrode montages from study 2 and study 3.

The current flow analysis was carried out to determine whether the montages used in studies 2 and 3 were stimulating different parts of the brain as anticipated. As shown in figure 8, the two montages had differential impacts on the areas of the brain getting stimulated, showing that this is a likely reason for the difference in results found between the two studies. With the montage used in study 3, the effects of stimulation remained mostly confined to the right hemisphere with the maximum current over the posterior parietal area. The current flow analysis of the montage used in study 2 showed effects crossing into the left hemisphere with a maximum current over superior parietal areas. As posterior parietal areas are more closely linked to visual working memory than superior parietal areas (e.g. Todd & Marois, 2004) this provides another possible explanation for the lack of a significant effect found in study 2.

However, the current flow analysis also indicates that the effects in study 3 extended to more anterior regions, including the right prefrontal area, relative to study 2. This supports the theory that stimulation of more anterior regions (including but not limited to the prefrontal cortex) may be responsible for the effects found in study 3. However, there is evidence to suggest that this is not the case from an unpublished study conducted as part of an undergraduate final year project (Romei, 2018, personal communication). This study used the same behavioural task as studies 2 and 3 but with a different electrode montage. Electrodes were placed over location Cz and the right supraorbital area. Again, stimulation at 4Hz and 7Hz was carried out along with a sham stimulation condition. This study produced no significant effects of stimulation in either of the active conditions. The results from this suggest that stimulation of anterior regions was not the reason for the significant findings of the montage used in study 3 compared to the montage used in study 2 but that, in line with previous evidence regarding the role of parietal theta activity in WM activity (e.g. Polania et al., 2012; Zakrzewska & Brzezicka, 2014), parietal stimulation was responsible for the modulation of WM performance in study 3.

7.4.5 Summary of the results from studies 2 and 3

In summary, the results from studies 2 and 3 provide support for the theta-gamma phase coupling theory of working memory whilst highlighting the importance of electrode placement in tACS studies.

The findings from study 3 suggest that, in line with the theta-gamma theory of WM (Lisman and Idiart, 1995), low parietal ITFs can increase WM capacity and high parietal ITFs can decrease WM capacity. What is more, there is a hemispheric specificity to this, with ITF in the right hemisphere only affecting visuospatial WM capacity for items presented in the left visual hemifield (these studies did not test for the converse hemispheric stimulation, therefore it cannot be extrapolated from this study alone that left parietal ITF effects visuospatial WM capacity for items presented in the right visual hemifield).

The contrast between the findings of studies 2 and 3 highlight the importance of electrode montage in tACS studies, particularly in regards to the potential desynchronisation of relevant neural networks or in regards to current flow distribution in relation to the area of interest. Importantly, the major finding from these studies, specifically from study 3, and the main aim of this thesis, is the evidence that off-peak entrainment can successfully occur and can be used to modulate the frequency peaks of neural oscillations (with the evidence in this particular case taking the form of behavioural evidence that parietal ITF was modulated through off-peak theta tACS). The implications of this finding will be discussed in more detail in the discussion chapter.

7.5 The role of gamma activity in working memory capacity

These studies explore the causal role of theta in the theta-gamma theory of WM capacity. However, a similar hypothesis about the causal nature of gamma peaks on WM capacity can be made from Lisman and Idiart's (1995) theory, with lower gamma peaks being predicted to decrease WM capacity and higher gamma peaks being predicted to increase WM capacity. A further study was conducted with this in mind, using the same behavioural task and protocol as in studies 2 and 3 but with active stimulation set at two different gamma frequencies. This study will be outlined in the following chapter.

Chapter 8: Study 4:

The modulation of working memory capacity through off-peak gamma stimulation

8.1 Introduction

From the theta-gamma phase coupling theory of WM capacity (Lisman and Idiart, 1995) outlined in the preceding chapters, predictions can be made regarding both theta frequency peaks and gamma frequency peaks and their impact on WM capacity. The results from study 3 supported the high-low theta peak prediction aspect of this theory, yet the impact of gamma peaks was unexplored. In order to further expand on the findings in relation to the gamma aspect of the theta-gamma working memory theory, a new study was conducted using the same behavioural task but with stimulation in the gamma frequency range. Again, a higher and lower variant of gamma frequencies were contrasted against each other and relative to sham stimulation. This follows the prediction of the theta-gamma phase coupling theory that changes in gamma peaks would affect WM capacity, with lower gamma frequencies meaning less gamma cycles could be nested in one theta cycle and higher frequencies allowing more gamma cycles to be nested.

Gamma activity has previously been linked to WM performance. Gamma bursts have been linked to both encoding and decoding of items (e.g. Lundqvist et al., 2016) and studies that have compared gamma activity during the presentation of successfully recalled items with unsuccessfully recalled items have demonstrated that bursts of gamma power are associated with successfully recalled items relative to unsuccessfully recalled items (e.g. Yamamoto et al., 2014). Gamma tACS has been found to enhance WM performance (Hoy, Bailey, Arnold, Windsor et al., 2015) and additionally, as mentioned previously, studies have shown that an increase of theta-gamma phase coupling correlates with increased WM load (e.g. Kaminski et al., 2011; Sauseng et al., 2009).

The category of gamma contains oscillations covers all frequencies above 30Hz. However, functionally it is often subdivided into low gamma and high gamma (although the frequency ranges of these subdivisions vary across the literature). Activity in the lower end of the gamma range had been previously linked to phase coupling with theta during WM tasks (e.g. Sauseng et al. 2008, 2009, 2010) and therefore this sub-range of gamma was used for the current study, with the frequencies of 40Hz and 70Hz being used as the low and high stimulation frequencies within the low-gamma subcategory.

8.2 Method

8.2.1 Participants

12 participants (9 female) took part in the study. Participants gave written consent to take part and completed a tACS safety screening questionnaire before each session. The study was approved by the local ethics committee.

8.2.2 Design

As in studies 2 and 3 a repeated measures design was used with the within subjects factors being stimulation condition, hemifield and memory load. There were two active stimulation conditions (40Hz and 70Hz tACS) and one control condition (sham stimulation) and these stimulation conditions took place during three separate sessions that occurred on separate days with the order counterbalanced across participants. At least 24 hours passed between different sessions. As with study 3, a single blind design was used, with the participants unaware that different stimulation protocols were being used for each session and unaware that one session consisted of sham stimulation. Again, this was due to the high likelihood of visual disturbances occurring with the particular montage used, the resultant necessity to adjust the power of stimulation for some participants and the limitations of the stimulation device to do this whilst following a double-blind procedure (see method section for study 3 for further details). As with study 3, a single blind design was considered appropriate.

8.2.3 Stimulation protocol

Two 5x7cm electrodes were placed over the scalp using saline soaked sponges (100mM solution). Prior to electrode placement skin was prepped with alcohol wipes to improve conductivity. One electrode was placed over the right parietal cortex (corresponding to P4 according to the 10-20 system; see Klem et al., 1999) and the other was placed on the forehead above the right eyebrow (supraorbital). Stimulation was delivered using a Magstim DC-Stimulator Plus device.

During the first session, stimulation at 1500μA was tested to see if retinal phosphenes or flicker occurred. If none occurred, this level of stimulation was used. If retinal phosphenes or flicker occurred at this level, the individual threshold for visual disturbances was determined for that participant. This was done by starting at 1000μA and increasing in increments of 25μA or decreasing in increments of 100μA and then back up in increments of 25μA until the highest level that did not produce retinal phosphenes was found. The average level of stimulation was 1255μA (±246μA). For the second and third sessions the voltage determined in the first session was used. In four cases, visual disturbances were reported at this level during the second or third session and the voltage was therefore reduced for the remaining sessions for those four participants (using the method outlined above to determine the appropriate level). In one case there was no resulting difference in voltage between the two active stimulation sessions (the only difference being between the initial sham stimulation session and the following active stimulation sessions). The three cases where there were voltage differences between the active stimulation sessions resulted in differences of +500μA, +400μA and +400μA for the 70Hz session relative to the 40Hz session. No other participants reported visual disturbances at the threshold determined during the first session.

As with studies 2 and 3, all conditions consisted of a ramping up stage at the beginning of the stimulation. For the 40Hz condition this lasted for 1200 cycles, for the 70Hz condition this lasted for 2100 cycles and for the sham condition this lasted for 1650 cycles. For the 40Hz and 70Hz conditions this was followed by stimulation at the predetermined threshold or at 1500μA, for the sham condition the ramping up period was followed by stimulation for 1650 cycles and then a cooling down period that lasted 1650 cycles. The brief stimulation period in the sham condition was carried out to mimic any sensations that may have been felt during the active stimulation conditions. This also allowed the retinal phosphene threshold to be determined when the first session contained sham stimulation. During the ramping up, stimulation and cooling down periods in the sham condition, the frequency of stimulation was at 55Hz. This frequency was chosen as a neutral frequency relative to the two active conditions. Throughout all stimulation sessions, impedance levels were kept below 5kΩ.

8.2.4 – 8.2.5 Materials and procedure

The materials, behavioural task and experiment procedure were the same as in studies 2 and 3 and will be outlined below.

8.2.4 Materials

A variation of the visual delayed match to sample task based on Vogel and Machizawa (2004) was used. Two arrays of coloured squares were situated on either side of a white fixation cross in the centre of a black screen. The number of squares in each array (the memory load) was 4, 5 or 6. The left and right arrays were always different from each other in any given trial; however, the number of squares in each trial was always the same for the left and right arrays. See figure 9b.

8.2.5 Procedure

Behavioural task

The task started with a fixation cross on the screen. Prior to presentation of the arrays, an arrow briefly appeared on the screen (200ms) to indicate which of the two upcoming arrays (left or right) needed to be memorised. The two arrays (one on the left and one on the right of the fixation cross) then appeared on the screen for 100ms. This was followed by a retention interval of 900ms during which there was a fixation cross on the screen. After the retention interval, two arrays appeared on the screen for 2000ms (again, there was one array either side of a fixation cross). See figure 9a. Participants had to indicate whether this array on the previously indicated side was the same or different to the array on that side presented previously during that trial. This was done by pressing either the left or right button of the mouse (left to indicate that the arrays were the same, right to indicate that they were different). The order of trials within each block was randomised by the computer program.

There were 6 blocks of 20 trials presented during each stimulation session. Each block contained trials of one load only (4, 5 or 6). For each session, the blocks were chosen at random from a set of 9 blocks (3 for each load) with the following restrictions: exactly 2 blocks of each load were to be used and no block was to be repeated during any given session. These 6 blocks were presented in a randomised order. Each block contained 5 left mismatched, 5 left matched, 5 right mismatched and 5 right matched trials.

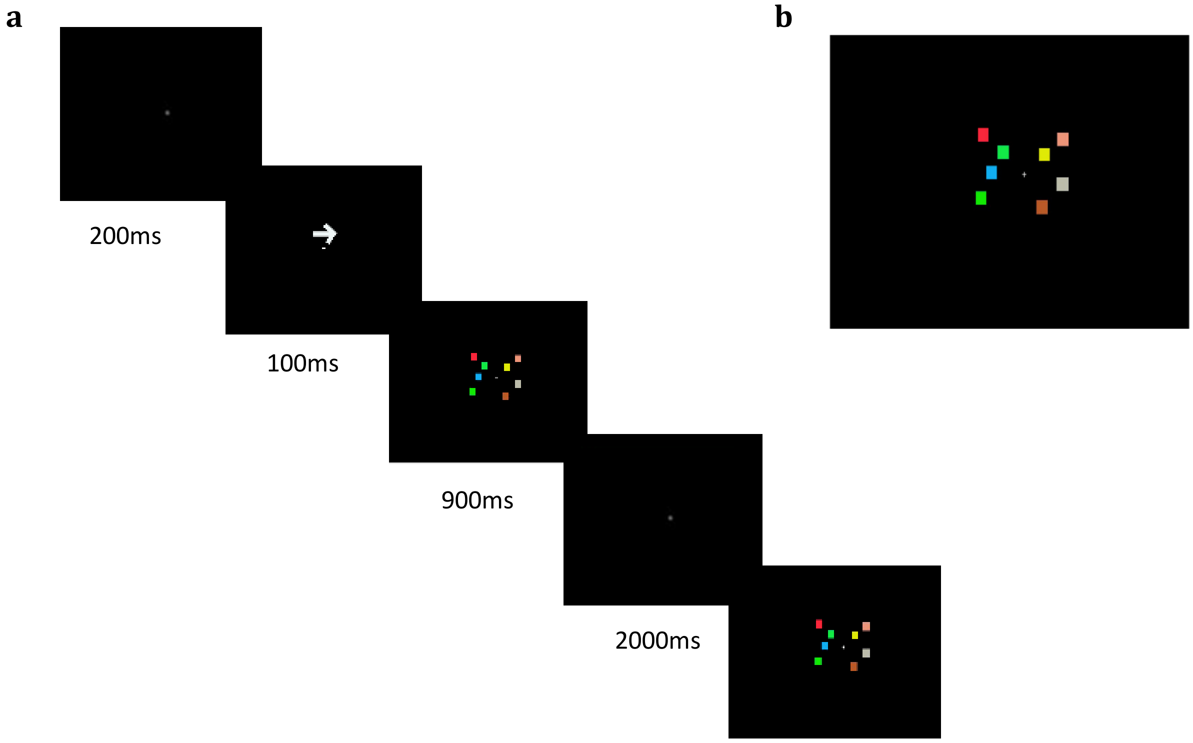

Figure 9. (a) Behavioural task. (b) Example of stimulus from a memory load 4 trial.

Experiment procedure

After the set-up of the electrodes, participants were presented with written instructions. Participants were instructed to use only one hand to make their responses and were given the opportunity to clarify the instructions if needed. The lights were turned off and participants carried out a practice block of trials. This practice block used load 3 stimuli to prevent load-specific practice effects affecting subsequent performance.

After the practice block had finished, additional saline solution was added to the sponges and stimulation was started. Participants were then instructed to start the first block when ready. After each experimental block, participants pressed the enter button to continue to the next block. Each block lasted approximately 2 minutes. Therefore, the total task time during stimulation lasted approximately 12 minutes. Participants were given monetary compensation or course credits for their participation after each of the three sessions and were debriefed after the final session and invited to ask any questions.

8.3 Results

8.3.1 Pre-processing of data into k values

The raw behavioural data was processed into k values (a standardised measure of WM capacity; see e.g. McCollough et al., 2007; Sauseng et al., 2009; Vogel et al., 2005; Vogel & Machizawa, 2004) for each combination of load x hemisphere x session per participant. This resulted in a total of 18 k values per participant (6 values per stimulation session). These values were calculated with the formula: k = (hit rate – false alarms) * set size/10.

8.3.2 Repeated measure ANOVA

A repeated measure ANOVA was conducted with the between subjects factors stimulation (40Hz, Sham and 70Hz), load (4 items, 5 items and 6 items) and hemifield (left and right). Means and standard deviations for each stimulation condition were also calculated (see table 6). There were significant main effects of load ($F(2,22) = 9.67$, $p < .001$, $\eta_p^2 = 0.47$) and hemifield ($F(2,22) = 5.56$, $p =.038$, $\eta_p^2 = 0.34$). Inspection of the means for load showed that, as expected, load 4 had the highest mean k value and load 6 had the lowest mean k value (mean k values; load 4 = 1.85 (sd. 0.77), load 5 = 1.28 (sd.

0.49), load 6 = 1.14 (sd. 0.62)). Post hoc paired sample t-tests revealed that there was no significant difference in k values between loads 5 and 6 (*t* = 0.88, *p* = .398, *r* = 0.26) and load 4 was significantly different from both load 5 (*t*= 3.24, *p* = .008, *r* = 0.70) and load 6 (*t* = 3.92, *p* = .002, *r* = 0.76). Analysis of the sham only trials showed a marginal main effect of load (*F*(1,22) = 2.96, *p* =.073, η_p^2 = 0.21).

	Mean	Std. Deviation
40Hz	1.35	0.74
Sham	1.53	0.62
70Hz	1.39	0.63

Table 6. Mean k values and standard deviations for each stimulation condition.

Comparison of the mean k values across the two hemifields revealed that k values were higher for left hemifield trials (mean k for left hemifield trials = 1.60 (sd. 0.59); mean k for right hemifield trials = 1.25 (sd. 0.60)). No interactions reached significance.

As mentioned in the method section, for three participants the voltage during 40Hz stimulation was less than the voltage during 70Hz stimulation. To ensure this did not confound the results, the data for these three participants were removed and the repeated measures ANOVA was run again. As with the initial analysis, main effects of load and hemifield were found (Load: *F*(2,16) = 6.60, *p* =.008, η_p^2 = 0.45; Hemifield: *F*(2,16) = 7.26, *p* = .027, η_p^2 = 0.48) but no interactions reached significance.

8.4 Discussion

The results from this study suggest that, in this instance, gamma stimulation was not successful in modifying WM capacity. However, theoretical extrapolations from the theory by Lisman and Idiart (1995) suggest that gamma does play a causal role in WM capacity. Possible reasons for the inconsistency between this theory and the current data will be outlined below.

8.4.1 The montage may not have been optimal

The positioning of the electrodes may not have been optimal for the modulation of the gamma activity involved in Lisman and Idiart's theta-gamma theory of WM capacity. It was expected that the best place for cortical stimulation of this specific gamma activity would be around the right parietal area, as the parietal area has been identified as a place where WM related theta-gamma phase-locking occurs (Sauseng et al., 2009). However, gamma activity in the prefrontal cortex has also been linked to WM activity (e.g. Alekseichuk et al., 2016; Bai et al., 2016; Hoy, Bailey, Arnold & Fitzgerald, 2015; Lundqvist et al., 2016) therefore the placement of an electrode at the supraorbital site may have caused a potential confound by additionally stimulating the prefrontal cortex at off-peak gamma frequencies. As the potential to cause confounds through the stimulation of other cortical areas was identified in the comparison between studies 2 and 3, the potential impact of this placement of the secondary electrode needs to be considered.

8.4.2 The frequencies may not have been optimal

In this study the frequencies of 40Hz and 70Hz were used as low and high frequencies within the gamma range of interest (the gamma range associated with theta-gamma phase coupling during a WM task). These frequencies did not appear to produce any significant changes in performance during the WM capacity task. Whilst these frequencies were chosen as lying on the outer ranges of this phase-coupled gamma range, it may be the case that 40Hz was too low for this particular range. Despite evidence that 40Hz is within the theta-gamma phase-coupling range (Sauseng et al., 2008), Sauseng et al. (2009) identified 50Hz-70Hz as the average gamma range involved in theta-gamma phase-coupling during WM activity. Therefore, 50Hz may have been a more appropriate low frequency to use in this experiment. However, the upper frequency of 70Hz corresponds to the findings of Sauseng et al. (2009) and individual t-tests in study 4 showed no difference in WM performance between the 70Hz and sham stimulation conditions. Therefore, despite physiological evidence suggesting that 70Hz may be an optimal frequency to use in off-peak gamma entrainment during a WM task, no evidence of any alteration of WM performance was found during 70Hz stimulation. Whilst a stimulation condition of 50Hz would be a useful comparison condition to have, the results of the 70Hz condition suggest that the range chosen was not the reason for no significant results being found for the higher gamma frequency condition at least.

Although 40Hz and 70Hz are within the theta-gamma phase-coupling range (Sauseng et al., 2008) (although see above for points made about whether 40Hz was optimal for this particular study), it may be the case that the frequencies used in both directions were too far removed from the average theta-coupled gamma frequency for the majority of participants. If the average gamma frequency for optimal theta-gamma phase-coupling during a WM task is approximately the mid-point between the range identified by Sauseng et al. (2009), i.e. 60Hz, then the stimulation frequencies would have deviated from this by an average of 10Hz and 20Hz in the 70Hz and 40Hz stimulation conditions respectively (alternatively, if using the range identified in Sauseng et al., 2008, both active stimulation conditions would have deviated from this midpoint by 15Hz). These frequencies may have been too far removed from the endogenous parietal gamma frequency peaks to be effective at modulating the frequency of gamma activity through off-peak entrainment. Or, alternatively, gamma activity around the stimulation frequencies may have become entrained but it may have been the case that these frequencies were too far removed from the optimal theta-gamma phase-coupled frequency for any behavioural changes to take place regarding WM capacity.

Since study 4 was conducted, a study by Alekseichuk et al., (2016) suggested that the range of 80Hz - 100HZ is the optimum range of gamma frequencies in the prefrontal cortex for theta-gamma phase coupling during working memory activity. Using this frequency range for gamma tACS applied to the prefrontal cortex, Alekseichuk and colleagues were able to successfully modulate WM performance.

The combination of findings from Alekseichuk et al. (2016) and Sauseng et al., (2008, 2009, 2010) suggests that the frequencies of theta-nested gamma oscillations involved in WM may differ across cortical regions, with lower gamma frequencies being involved in parietal theta-gamma phase coupling and higher frequencies of gamma (80Hz-100Hz) being involved in prefrontal regions. Whilst parietal stimulation at lower gamma frequencies was the primary focus of the current study, prefrontal regions were also likely stimulated due to the electrode montage used. As highlighted above, prefrontal stimulation may have caused a confound. Additionally, in light of the findings from Alekseichuk et al., (2016), the fact that the frequency of such prefrontal stimulation may not have been optimal causes a further potential confound.

It is clear from the above that more research would be useful in order to determine the optimal gamma frequencies to use across different cortical areas when attempting to modulate WM performance through off-peak gamma stimulation.

8.4.3 Gamma stimulation may need to be timed to theta peaks

In the study by Alekseichuk et al., (2016), gamma bursts timed to theta peaks, but not theta troughs, were found to correlate to improved WM performance. In the current study, gamma tACS was not locked to theta activity and was delivered as a constant sine wave rather than in bursts. Therefore, it may be the case that the type of gamma stimulation used, along with its timing, was not optimal for improving WM performance. Gamma burst stimulation delivered during theta peaks may be a more optimal type of gamma stimulation for improving WM performance.

8.4.4 Low gamma frequencies over parietal areas may not causally effect WM capacity

There is theoretical evidence that parietal gamma frequencies causally effect WM capacity (Lisman and Idiart, 1995) and there is empirical evidence that WM performance is causally effected by parietal theta frequency peaks (Study 3; Vosskuhl et al., 2015; Wolinski et al., 2018) and by prefrontal gamma activity nested into theta peaks (Alekseichuk et al., 2016). However, this study did not find empirical evidence that parietal gamma frequencies play a causal role in WM capacity. Whilst potential methodological reasons for this were raised in sections 8.4.1 – 8.4.3 (and further studies investigating this would be useful) it may be the case that, despite theoretical support, modulation of peak gamma frequencies over parietal areas does not causally effect WM capacity.

8.4.5 Areas for further investigation

The current study relied on behavioural results to indicate whether off-peak entrainment had occurred. Future studies using concurrent physiological recordings (e.g. MEG or EEG with an artifact removal algorithm) would help to clarify whether frequency modulation has taken place. This would indicate whether the lack of WM capacity modulation was the result of no frequency modulation taking place or whether instead, frequency modulation occurred but not in a form that was effective at modulating WM capacity. If frequency modulation was found to occur from these physiological recordings, a follow-up study comparing different off-peak gamma frequencies and different electrode montages may then be beneficial in helping to determine whether low gamma frequency peaks in parietal regions can causally effect WM capacity in a way that mirrors the impact of theta frequency peaks found in study 3.

8.5 Conclusion

In conclusion, this study did not lend support to the theory that gamma frequency peaks can causally affect WM capacity. However, this study was not a comprehensive investigation into this area as no physiological recordings were taken and only a limited range of parameters were explored. As study 3 demonstrated successful modulation of WM capacity through off-peak theta stimulation, and prefrontal theta-linked gamma tACS has been shown to successfully modulate WM performance (Alekseichuk et al., 2016), further studies exploring the potential ways in which gamma activity plays a causal role in WM capacity may be useful. Further investigations exploring more parameters (i.e. a wider variety of off-peak frequencies and alternative montages), alongside concurrent physiological recordings, would be a potential way of exploring these questions further.

The next chapter will summarise and discuss the findings and implications from the four studies in this thesis and will outline any outstanding questions that could potentially form the basis for further research.

Chapter 9: Discussion

This thesis aimed to investigate both the behavioural and electrophysiological effects of transcranial neurostimulation delivered at non-peak frequencies. The studies looked at off-peak frequencies within the alpha band (study 1), theta band (studies 2 and 3) and low-gamma band (study 4), with all studies comparing frequencies that could be considered low and high within their respective bands (or sub-band in the case of study 4). These studies aimed to answer questions about the effects of stimulation at different frequencies, with a particular focus on whether stimulation at off-peak frequencies (i.e. frequencies that were non-dominant or non-average within a particular frequency band) could affect the dominant or average frequency of the respective frequency band and how this might affect behaviour.

9.1 Overview of studies

9.1.1 Study 1

General overview

In the first study, tACS was delivered at off-peak alpha frequencies. These frequencies were tailored to each individual participant and were delivered at both 2 Hz above and 2Hz below the IAF of each participant across two different sessions (a sham stimulation session was included as a control). EEG was recorded up to and immediately following the tACS stimulation and then analysed for any changes in peak frequencies following the active tACS sessions relative to sham stimulation. No significant changes in frequency peaks were found for either condition relative to sham.

The non-significant findings suggest that tACS delivered at the parameters used was not effective at altering IAF in offline EEG recordings in this instance. It could be the case that the parameters used are not effective in altering offline IAF generally. Due to the fact that only two off-peak frequencies were used in this study, IAF +2Hz and IAF -2Hz, (given the practical restraints of using a larger range of frequencies within this PhD) it is not possible to extrapolate to off-peak alpha stimulation in general. A larger scale study, comparing off-peak alpha stimulation across a large range of frequencies relative to the IAF may provide a clearer idea as to whether off-peak alpha stimulation is not effective in offline IAF manipulation per se or whether some off-peak frequencies are more effective at this manipulation than 2Hz above and below IAF.

Interestingly, over the course of three stimulation sessions per participant, it became clear that IAF is not consistent over even a short space of time, even when a person is conducting the same task (in this case, relaxing with eyes open). Further research into this phenomenon would be an extremely useful avenue to explore. It would be interesting to explore this area in terms of questions relating to stimulation such as whether the effectiveness of tACS manipulation varies depending on the stability of the individual's IAF and also with questions that do not relate to stimulation, such as are there certain trait, cognitive or behavioural correlations associated with more or less stability over time in a person's IAF.

As well as individual differences in stability, the spectral analysis power graph varied widely across participants, with some showing a clear singular peak in the alpha bandwidth, and others showing multiple smaller peaks around a dominant peak (and one participant, whose data was excluded due to there being two dominant alpha peaks at different ends of the spectrum). Chiang et al. (2011) started investigating the phenomenon of split alpha peaks, including investigating the hypothesis of whether single alpha peaks are in fact split peaks which are close together in frequency. Studies investigating whether these differences in alpha peaks affect the effectiveness of frequency manipulation through tACS would be a further potential avenue to explore.

Limitations of this study

Due to the technical constraints of concurrent EEG and tACS, the first study was only able to investigate off-line effects of tACS (in this case, the minutes immediately preceding the stimulation). It was therefore possible that the lack of significant results was due to the off-line nature of the task. In the absence of online physiological recordings, it would not be possible to extrapolate from the offline results to form any conclusions about whether or not entrainment successfully occurred during the actual stimulation. Therefore, the possibility that off-peak alpha stimulation at IAF +/-2Hz successfully altered IAF in this instance cannot be ruled out as online EEG data were not recorded. Recent algorithms are making the possibility of online EEG recordings possible during tACS, however, the issue of data contamination is not completely erased with such methods. In order to address this limitation of the first study, instead of physiological EEG recordings, the subsequent studies used a behavioural measure of frequency change (one with a robust theoretical link to different frequencies within the theta and gamma ranges with further empirical support for this link within the theta range) which was able to be carried out during stimulation.

9.1.2 Brief summary of the theta-gamma theory of WM

The behavioural measure used in all subsequent studies (studies 2, 3 and 4) was the performance in a visuo-spatial working memory task (based on the task used by Vogel and Machizawa, 2004). This task capitalised on the theta-gamma theory of working memory capacity posed by Lisman and Idiart (1995). When taken to its logical conclusion, Lisman and Idiart's theory has a clear prediction regarding individual differences in theta and gamma frequencies and individual differences in working memory capacity. This lends itself well to neurostimulation studies that aim to manipulate theta and gamma frequencies. Specifically, the theory predicts that lower individual theta frequencies (ITF) lead to increased WM capacity and higher ITF lead to decreased WM capacity. The converse is predicted for individual gamma frequencies (IGF).

Alternative models of working memory

Lisman and Idiart's theory is based on a slot model of working memory where there are discrete slots for memory items (e.g. Luck & Vogel, 1997). Whilst there is support for this model (e.g. Awh et al., 2007, Donkin et al., 2013; Luck & Vogel, 2004; Vogel & Machizawa, 2004) there is also support for a continuous, flexible model of working memory (also referred to as a resource-based model) (e.g. Bays, 2015; Bays et al., 2009; Ma et al., 2014). The empirical findings of study 3 correspond to the theoretical expectations from Lisman and Idiart's slot-based theory. However, it must be noted that this study does not provide any direct evidence that the results are due to an increase or decrease in the number of gamma 'slots' and the finding that theta frequency can effect WM does not contradict a resource-based model of WM. As the findings of study 3 have been interpreted from within the framework of a specific slot-based theory of working memory, caution must be used when extrapolating these findings to then provide evidence for a slot-based model in general.

9.1.3 Studies 2 and 3

General overview

The first two behavioural studies in this thesis (studies 2 and 3) focused on modulating ITF to bring it towards the lower end of the theta range in one condition (low theta) and towards the higher end of the theta range in another condition (high theta); a sham stimulation condition was also used for comparison. The studies used two different electrode set-ups but remained consistent across all other variables. Both electrode montages focused on the right parietal region, with an electrode positioned over electrode site P4 using the 10-20 system (see Klem et al., 1999). The other electrode was positioned over the vertex in study two and over the right supraorbital area in study three. Study 2 showed no significant differences in WM performance across the low theta and high theta conditions relative to sham stimulation. However, study 3 showed a significant effect of stimulation condition, with WM performance altering in line with the predictions of Lisman and Idiart's theory. Furthermore, these changes in WM capacity only took place for the trials presented in the visual hemisphere ipsilateral to the stimulated hemifield (i.e. the left visual field) site, suggesting that the stimulation effects reflected the hemispheric specificity of the visuospatial WM processes used.

The findings from study 3 supported the Lisman-Idiart theory of WM capacity and added support for a causal element of this theory, with theta frequency playing an active role in WM capacity rather than being merely an epiphenomenon. The comparison of studies 2 and 3 allowed insights to be gained regarding electrode placement during tACS studies. In both studies, the main site of interest was the right parietal area over site P4 on the 10-20 system. An electrode was placed over this site in both montages yet only one montage produced significant changes in WM performance. This highlights the importance of the secondary electrode site during tACS studies. Often in tACS studies, there is only one site of interest for stimulation (as was the case with studies 2 and 3). In such cases it could be tempting to place the secondary electrode merely out of the way of the site of interest. However, as tACS creates a circuit through cortical areas this must be taken into account when positioning both electrodes regardless of whether one is merely the 'other' electrode. Placement of this secondary electrode must be considered carefully in order to minimise disruption along neural pathways of interest or neural pathways that could potentially confound the results. Possible desynchronization across the neural pathway being investigated is a concern (and could possibly explain the results of study 2 in this case). Another concern to be avoided if possible, is generic stimulation across an additional neural pathway whose heightened activity could potentially influence the results (a simple example of this would be stimulation of a visual pathway during a cognitive task that uses visual stimuli).

Limitations of these studies

No EEG recordings were taken during these studies. As indicated in the summary of study 1, frequency analyses of offline EEG recordings were specifically avoided for these studies as these studies focused on potential online changes. It has also been explained why online EEG recordings were not feasible. However, carrying out initial EEG recordings to identify individual theta frequencies for each participant could have allowed subsequent stimulation to be tailored to each individual. This was not done due to the time constraints involved. If time had not been limited, such EEG recordings would have ideally been carried out whilst the participants performed the behavioural task to ascertain their average theta frequency whilst visuospatial WM was being specifically engaged. The calculation of the ITF would have focused on the frequencies around the parietal site of stimulation (i.e. the area around P4 using the 10-20 electrode placement system). This would be to ensure that the ITF related to the particular theta oscillations of interest to the study (parietal oscillations in the frontoparietal theta pathway). During study 1, IAF varied within participants across the different EEG sessions. The same would not necessarily be the case for ITF, however, based on this finding of the first study, it would be ideal to carry out this preliminary EEG recording before each stimulation session if such a study were to be conducted in the future. Whilst the findings were significant, tailoring the stimulation frequencies in this way would possibly have made the findings even more robust. Inter-individual variability of the effectiveness of the stimulation (i.e. how much WM performance was worsened or improved compared to sham) may have been more consistent as it was likely that, with the generic low and high frequencies of 4Hz and 7Hz used, some participants were being stimulated far closer to their ITF than others during one of the active stimulation sessions. For a more sophisticated method of tailoring the stimulation frequencies to the individual, the optimal theta frequency for theta-gamma phase-coupling could be used as a starting point rather than absolute ITF as the theory behind this study focuses on the interplay between theta and gamma oscillations as opposed to focusing on theta frequency in isolation.

9.1.4 Study 4

General overview

In the same way that Lisman and Idiart's theta-gamma WM theory (1995) suggests that individual theta frequencies may determine WM capacity, it can be extrapolated that individual gamma frequencies may similarly determine WM capacity. The fourth study of this thesis investigated this proposal. It is notable that there is less support from the literature for differences in IGF corresponding to differences in WM capacity. Furthermore, there are less cases of gamma frequencies being successfully modified through frequency-based neurostimulation (although see, for example, Alekseichuk et al., 2016). Nevertheless, in light of the successful modulation of WM performance through theta frequency modulation, a corresponding study was carried out attempting to modulate gamma frequencies.

This study found no significant changes in WM performance when stimulating at frequencies that corresponded to the lower and higher ends of the low gamma frequency range (40Hz – 70Hz). These findings may reflect that parietal gamma frequency does not play a causal role in the way that parietal theta frequency appears to. Alternatively, especially in light of the different results between studies 2 and 3, it could be the case that for gamma activity, the particular parameters used in study 4 (such as electrode montage and stimulation frequencies) were not optimal for the modulation of WM performance in this case. Further work is warranted to explore these possibilities.

Limitations of this study

As with studies 2 and 3, EEG recordings prior to the stimulation would have allowed the low and high gamma frequencies used during the tACS sessions to be more tailored to the individual.

9.2 Contributions to the current literature

The main areas in which the studies in this thesis contributed to the current literature will now be outlined.

9.2.1 Methodology of tACS studies

The differences in the effectiveness of stimulation between studies 2 and 3 highlight the importance of montage during tACS studies. Both studies had the same main area of interest (right parietal) and therefore both montages included an electrode placed over site P4. However, the second electrode in the montage differed across the studies and the subsequent results varied, with only the montage used in study 3 being effective at modulating WM capacity. This contrast indicates that If there is only one area of interest in a tACS study, careful consideration must be given to the placement of the other electrode in order to reduce possible contamination resulting from stimulation over the second electrode. In the case of study 2 it was expected that this contamination may have taken the form of desynchronization across the network of interest. However, even in studies where this is irrelevant or avoided, other confounds can occur as there will always be superfluous brain regions which will receive indirect stimulation as a result of the current flow between the electrodes. In light of this, a current flow analysis, alongside careful consideration of which brain regions would be most likely to confound the current study if stimulated, would be good practice during the design phase of tACS studies.

Studies 2,3 and 4 also indicated that, through utilising behavioural predictions from a robust and empirically supported theory, behavioural experiments can be used to circumnavigate the practical issues surrounding simultaneous stimulation and physiological measurements. Although caution should always be taken when interpreting such results.

Studies 1 and 4 did not produce significant results. From this, no conclusive suggestions could be made as only a small subset of potential parameters (e.g. stimulation frequency, electrode placement, stimulation intensity) had been investigated. This highlights current limitations regarding the understanding of the effects that these different parameters can have on off-peak entrainment through tACS. As this is a relatively new technique this is to be expected, however it does highlight the importance of investigating these methodological aspects further, both in order to aid experimental design and in order to allow for more confident conclusions to be made from non-significant results. For example, if it was known that optimal parameters had been used in study one, more definitive conclusions could have been made regarding the absence of after-effects in the EEG data. Similarly, in study 4, more definitive conclusions could have been made regarding the causal nature of gamma peaks in WM capacity.

9.2.2 Neural correlates of working memory

The theta-gamma working memory theory by Lisman and Idiart proposes that individual memory items are linked to individual gamma oscillations, which are nested within a theta cycle (with the theta cycle working as a binding mechanism). It therefore logically follows that longer theta cycles (relating to lower theta frequencies) would be able to bind more gamma cycles and vice versa. As each gamma oscillation is thought to correspond to one item in WM, the more gamma cycles able to be nested within a theta cycle, the higher the potential WM capacity would be, hence longer theta cycles (i.e. lower theta frequencies) would cause a larger WM capacity.

As low theta stimulation would be expected to decrease ITF and high theta stimulation would be expected to increase ITF (through off-peak entrainment, see below), a likely interpretation of the behavioural findings from study 3 would be that stimulation at 4Hz lowered theta peaks, leading to an increase in WM capacity, and stimulation at 7Hz increased theta peaks, leading to a decrease in WM capacity. This is in line with the prediction of Lisman and Idiart's theta-gamma theory of WM capacity (Lisman and

Idiart, 1995) that lower theta peaks correspond to higher WM capacity and higher theta peaks correspond to lower WM capacity.

The bi-directional findings from this study (showing that WM capacity can be both increased and decreased depending on theta frequency peaks), and the fact that these findings are causal in nature, provides a valuable contribution to the literature surrounding the neural correlates of WM, particularly regarding the role that brain oscillations play in WM capacity.

9.2.3 Off-peak entrainment

Whilst no physiological recordings were conducted in study 3, the behavioural findings indirectly suggest that rhythmic stimulation at off-peak frequencies can modify frequency peaks during the stimulation period (potential after-effects were not tested for during study 3 therefore it cannot be determined whether such effects can outlast the stimulation period). The most likely explanation for this modulation of frequency peaks is through entrainment. As entrainment causes endogenous brain oscillations to phase-lock with the external oscillator it would follow that frequency shifting of the endogenous brain oscillations would occur when the frequency of the endogenous and external oscillations do not match. Therefore, an increase in brain oscillations power (synchrony) at this frequency would occur. This increase in power would alter the frequency peak towards the new frequency.

As off-peak entrainment is a relatively new area of research within the entrainment literature, the evidence of its efficacy from study 3 can prove useful in guiding future research in this area.

An additional conclusion that can be made from study 3, which has practical applications for the future of off-peak entrainment research (alongside the future of entrainment research in general) is the demonstration that off-peak entrainment has effects which can be observed on a behavioural level. This has strong implications for the future use of off-peak entrainment, particularly regarding its potential as a therapeutic tool.

9.3 Directions for future research

9.3.1 Varying parameters

The studies in this thesis further support the idea that changes due to tACS are subtle and many variables may have an effect. Therefore, it is important to consider all parameters when designing a tACS study. The current tES literature uses a variety of different parameters to investigate a variety of different cognitive functions and physiological phenomena. A meta-analysis has been carried out to investigate some of the parameters used across tDCS studies (Imburgio & Orr, 2018), however, as the current literature regarding tES studies is very varied, it is difficult to pinpoint how changes across one parameter might be affecting the outcomes of stimulation. The studies in this thesis highlighted how future studies could take a systematic approach to varying the frequency of stimulation and investigating the effects. However, this is not limited to just the frequency of stimulation. The future of tACS (and tES in general) studies would benefit from a systematic analysis of the effects that occur when changing various parameters in multiple increments and directions. For example, a study that consistently keeps all parameters the same except amplitude of the stimulation, another study that only alters the electrode montage, another that systematically alters the frequency in small increments whilst keeping all other variables constant.

One aspect of tACS that has not been explored much is the application of different waveforms. The majority of tACS studies to date have used a sinusoidal rhythm. However, as highlighted by Dowsett and Herrmann (2016), alternative rhythms, such as a sawtooth rhythm, may be more suited to certain applications. A systematic investigation applying different waveforms to different cortical rhythms would shed more light on this relatively new area of tACS research. One particularly interesting avenue for exploration would be whether tailoring the waveform pattern of the stimulation to the waveform shape of the target rhythm (e.g. a wicket-like stimulation applied to the sensorimotor mu rhythm) produces more effective results than stimulation with a non-matched rhythm (e.g. a sinusoidal stimulation applied to the sensorimotor mu rhythm). Such in depth studies across the different parameters of tACS would allow for a better understanding of the nuances of tACS application and would be a crucial aide when designing future studies.

9.3.2 Online EEG-tACS

One limitation of the studies in this thesis was the lack of simultaneous EEG/tACS. This was due to the technical constraints of using both techniques simultaneously, as electrical noise from the tACS contaminates the EEG data creating strong electrical artifacts. By the nature of these studies, the tACS noise would have been at the frequency of interest for the EEG analysis, exacerbating any potential issues with electrical artifacts.

Very recent advances in this area have led to new algorithms being developed that allow for EEG recordings to be taken online during tACS stimulation (e.g. Helfrich et al., 2014). These algorithms are relatively new and their development has the potential to open up many possibilities for research into the physiological effects of tACS. All of the studies conducted within this thesis would benefit from online EEG recordings processed with tACS artefact removal in order to shed more light on the exact electrophysiological effects that the stimulation was producing and the relation of these effects to both concurrent cognitive/behavioural changes (in the case of the memory tasks) and to subsequent electrophysiological activity (in the case of the offline EEG recordings). In study one simultaneous EEG recordings would help to clarify whether changes had occurred and then ceased during the offline recordings or whether no changes had occurred in the first place. In the case of the study 3, changes in theta peaks were deduced from the behavioural data, but simultaneous EEG recordings would allow for a stronger conclusion to be reached regarding the link between the behavioural outcome and theta activity (alternatively such data could possibly provide evidence to contradict the theta-gamma theory). The improvement that online EEG recordings could make to these studies is indicative of how useful such recordings could be for tACS research in general. The successful use of concurrent EEG recordings during the tACS study by Helfrich et al., (2014) highlights the usefulness of research in this area and the potential that such studies have to further understand the physiological effects of tACS.

9.3.3 Therapeutic interventions

The finding that tACS at varying theta frequencies can affect WM performance has potential therapeutic connotations. Whilst the effects of single session tACS are temporary, wearing off after approximately 70 minutes (Kasten et al., 2016), there is evidence to suggest that repetition of tES sessions can produce effects that last beyond each session (e.g. Klimke et al., 2016; Mori et al., 2010). In relation to the studies in this thesis, such therapeutic potential could take the form of improving WM performance. This could be highly useful in the case of pathological memory impairments or in memory impairments associated with ageing. If regular tACS sessions stimulating at low theta frequencies could produce a prolonged improvement to WM capacity this could have a huge benefit to populations with WM impairments. It is worth noting that WM capacity could potentially be improved in non-pathological populations also, however, as, despite its name, there is an element of invasiveness to NIBS techniques (see Davis & van Koningsbruggen, 2013), the cost-benefit analysis of such an application would need to be carefully considered. Furthermore, consideration needs to be given to the ethical implications of the usage of neural enhancement in the absence of neuropathologies (see Erhardt & Strac, 2016; Nagel, 2014 and Voarino et al., 2016 for a general introduction to this ethical debate, along with Iwry et al., 2017, for a discussion of NIBS and personal identity and Lavazza, 2017, for an insight into issues of equality regarding NIBS enhancement).

However, it is clear to see the benefits that could be offered if regular low-theta tACS could be used to improve WM performance in populations with pathological WM impairment or age-related WM capacity decline. As mentioned in chapter 2, one of the major benefits of tACS over the other rhythmic stimulation technique of rhythmic TMS, is the fact that it can be self-administered at home as opposed to being administered by a specialist in a clinic or laboratory (e.g. see Martens et al., 2018). This has important practical connotations if the stimulation were to be used therapeutically as the technique would therefore be far more convenient and less intrusive to daily life for any potential patients. Furthermore, the cheaper cost of tACS compared to TMS, both in terms of equipment and staff/clinician contact hours, would also allow such a therapy to reach a wider range of potential patients. Whilst all tES techniques are considered safe for home application, tACS has an added safety benefit over tDCS regarding unsupervised application in that power build-up does not occur due to the changing direction of the current. Therefore, usage beyond the recommended time, whilst obviously highly not recommended, would not have the same risk that tDCS could pose. (Ideally though, safety limits would be built in to devices for at-home usage to prevent overuse, so such considerations would not be necessary).

In addition to demonstrating that tACS can successfully improve WM capacity, study 3 in this thesis also provided more general evidence that stimulation at a low theta frequency can improve WM capacity. Therefore, the implications for frequency-based therapies extend beyond tACS. Neural pacemakers have been successfully used to treat neurological disorders such as Parkinson's disease (e.g. Schuepbah et al., 2013), Tourette's syndrome (Viswanathan et al., 2012), depression (Accolla et al., 2016; Lakhan & Callaway, 2010) and OCD (Lakhan & Callaway, 2010). As the understanding of the role of theta frequencies in WM performance increases, such deep brain stimulation techniques may also become a potential avenue for future therapies.

It is important to note that the participants taking part in the studies within this thesis had no memory impairments. Therefore, any speculation as to the therapeutic potential of low theta stimulation is limited by this factor. Any future investigation into the possibility of these techniques being used in this way would need to start with research into whether the findings from study 3 extend to a population with WM impairment. If such a finding is established, then further studies looking at longer-term improvements in WM capacity (through regular stimulation sessions) with a view to creating stimulation-based therapies for populations with WM impairments would be a potentially useful area of future research.

The potential therapeutic applications for WM impairment have already been discussed. However, the fact that tACS was found to successfully modulate frequency-specific performance in a cognitive task has implications for additional therapeutic applications outside the area of WM where frequency modulation may also be beneficial. For example, individual differences in IAF have been linked to individual differences in cognitive abilities (e.g. Anokhin & Vogel, 1996; Clark et al, 2004; Klimesch, et al., 1990; Klimesch et al., 1993). Therefore, a potential avenue for exploration would be whether tACS could be used to modulate IAF in populations with impairments in these areas. The same potential for future research also applies to neuropathologies associated with altered activity in other frequency bands.

9.4 Conclusion

The findings from these studies, along with the current literature surrounding this area suggest that off-peak entrainment can be used to successfully modulate frequency peaks. The potential uses of such modulation include being able to research the causal nature of specific frequencies, particularly in the areas of individual differences in peaks within a given frequency band.

As indicated in chapter 1, individual differences in frequency peaks can correlate to neuropathologies, differences across the lifespan and also to different cognitive tasks (e.g. Anokhin & Vogel, 1996; Chiang et al., 2011; Dickinson et al., 2018; Haegens et al., 2014; Scally et al., 2018). Being able to modulate individual frequency peaks in an experimental setting and investigate the resultant effects would therefore be highly useful when further investigating such areas. Such modulation would allow studies to investigate the causal nature of differences in frequency peaks, as was the case with study 3. As well as adding to the general understanding of the role that specific aspects of brain oscillations play in cognitive functioning, experiments of this sort may prove particularly useful when researching relationships between differences in frequency peaks and certain neuropathologies (e.g. de Frutos-Lucas et al., 2018; Dickinson et al., 2018). Such modulation would allow for differences in frequency peaks to be investigated in a neurotypical population and therefore isolated from any comorbid differences in brain activity or behaviour that accompany the associated neuropathology.

The significant behavioural effects from study 3 indicate that frequency peak modulation can be successfully used as a research tool for investigating the effects that frequency peaks can have on cognitive functions. Additionally, the findings from studies 1, 2 and 4 highlight that this technique is in its infancy and would benefit from systematic research being carried out in order to establish the changes that can occur when subtle shifts to parameters are made. This direction, of carefully investigating the effects of changes across different parameter, would be an extremely useful next step in the area of off-peak entrainment research through NIBS as more in depth knowledge of these practical aspects of the technique (and the resulting effects) would help to guide future studies in this area.

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Appendix

Appendix 1:

Wolinski, N., Cooper, N. R., Sauseng P., & Romei, V., (2018) The speed of parietal theta frequency drives visuospatial working memory capacity*. PLoS Biology, 16(3),* e2005348

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SHORT REPORTS

The speed of parietal theta frequency drives visuospatial working memory capacity

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Abstract

The speed of theta brain oscillatory activity is thought to play a key role in determining working memory (WM) capacity. Individual differences in the length of a theta cycle (ranging between 4 and 7 Hz) might determine how many gamma cycles (>30 Hz) can be nested into a theta wave. Gamma cycles are thought to represent single memory items; therefore, this interplay could determine individual memory capacity. We directly tested this hypothesis by means of parietal transcranial alternating current stimulation (tACS) set at slower (4 Hz) and faster (7 Hz) theta frequencies during a visuospatial WM paradigm. Accordingly, we found that 4-Hz tACS enhanced WM capacity, while 7-Hz tACS reduced WM capacity. Notably, these effects were found only for items presented to the hemifield contralateral to the stimulation site. This provides causal evidence for a frequency-dependent and spatially specific organization of WM storage, supporting the theta–gamma phase coupling theory of WM capacity.

Author summary

Our ability to temporarily retain sensory information is limited to a handful of items and is referred to as working memory capacity. Such memory capacity has been shown to vary across the general population, with some people retaining a higher number of items than others. An influential theory suggests that this individual capacity might be determined by the speed of slow brain waves (so-called theta waves) that range in frequency between four and seven cycles per second. It is hypothesized that these theta waves act as glue for items to be memorised such that the slower the theta waves, the higher the number of items that can be clustered and retained in memory. We tested this hypothesis by applying to human participants noninvasive current stimulation at slower or faster theta frequencies over a part of the brain that is involved in visuospatial working memory during a visuospatial task. In line with this influential theory, we found that stimulation at slower theta frequencies enhanced working memory capacity relative to stimulation at faster theta frequencies, which instead reduced working memory capacity. These effects were limited to visual

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Abbreviations: EEG, electroencephalography; ERP, event related potentials; IPS, intraparietal sulcus; MEG, magnetoencephalography; NIC, Neuroelectrics Instrument Controller; tACS, transcranial alternating current stimulation; TMS, transcranial magnetic stimulation; WM, working memory.

items processed by the stimulated brain areas, confirming the importance of theta waves for the organization of visuospatial working memory.

Introduction

The theta–gamma cross-frequency coupling theory [1] (Fig 1A) proposes that individual fast brain waves (gamma cycles) represent individual memory items that are bound together to a multi-item memory by slow brain waves (theta oscillations). Consequently, individual differences in the length of a theta cycle (4–7 Hz) might determine how many gamma cycles (>30 Hz) can be nested into a theta cycle and may therefore determine memory capacity. This theory provides a potential neurophysiological mechanism for individual differences in the maximum number of items (number of gamma cycles) retained in the memory buffer (one theta cycle). According to this theory, it would be expected that slower theta frequencies will integrate a higher number of gamma cycles per theta cycle, resulting in increased memory capacity. Conversely, faster theta frequencies would bind a comparatively smaller number of nested gamma cycles, resulting in a decreased memory capacity.

Correlational studies have provided indirect support for this theory (e.g., [2,3]). For instance, Axmacher and colleagues [2] showed that increasing working memory (WM) load leads to a slowing down in the theta frequency. Moreover, recent neurostimulation work has shown that entraining parietal theta oscillations via transcranial alternating current stimulation (tACS) [4] or rhythmic Transcranial Magnetic Stimulation (TMS) [5] has proven effective in enhancing WM performance, providing causal evidence for the role of theta oscillations in WM performance. These works have so far mainly focused on enhancing theta amplitude by enhancing the theta signal-to-noise ratio, leading to better performance. A recent work has attempted to enhance WM capacity through manipulation of the intrinsic theta cycle length by frontal tACS set at a stimulation frequency slower than the individual theta [6]. However, it is unclear whether the enhanced WM performance obtained in that study is due to slowing of theta frequency or can be attributable to the more general impact of stimulation on theta amplitude, per se. Therefore, previous studies have left unanswered a long-lasting question regarding the exact mechanism by which theta oscillations orchestrate WM capacity: does the cycle length of theta oscillations play a mechanistic role in determining interindividual variability of WM capacity? Here, we test the prediction based on the theta–gamma phase coupling theory [1] that inducing slower theta cycles will enhance WM capacity, while inducing faster theta cycles will reduce WM capacity. We tested this prediction in a visuospatial WM paradigm based on seminal work by Vogel and Machizawa [7,8,9], who showed interindividual differences in visuospatial WM capacity to positively correlate with the amplitude of evoked responses localised over parietal areas contralateral to the hemifield where the stimulus to be kept in memory was presented. Crucially, using the same paradigm, Sauseng and colleagues [3] found a clear lateralisation of theta-locked gamma phase synchronization increase over parietal areas, again predicting individual WM capacity. Therefore, based on recent electroencephalography (EEG) [10], magnetoencephalography (MEG) [11], and behavioural evidence [12] that tACS can drive the intrinsic resonance frequency towards an externally imposed rhythm [13], we directly tested for the modulation of WM capacity by slow (4 Hz) and fast (7 Hz) theta tACS over parietal areas [3,7,8,9]. In line with our predictions, we found that slow theta tACS enhanced WM capacity while fast theta tACS reduced WM capacity. Importantly, these effects were specifically obtained for the visual hemifield contralateral to the stimulation site.

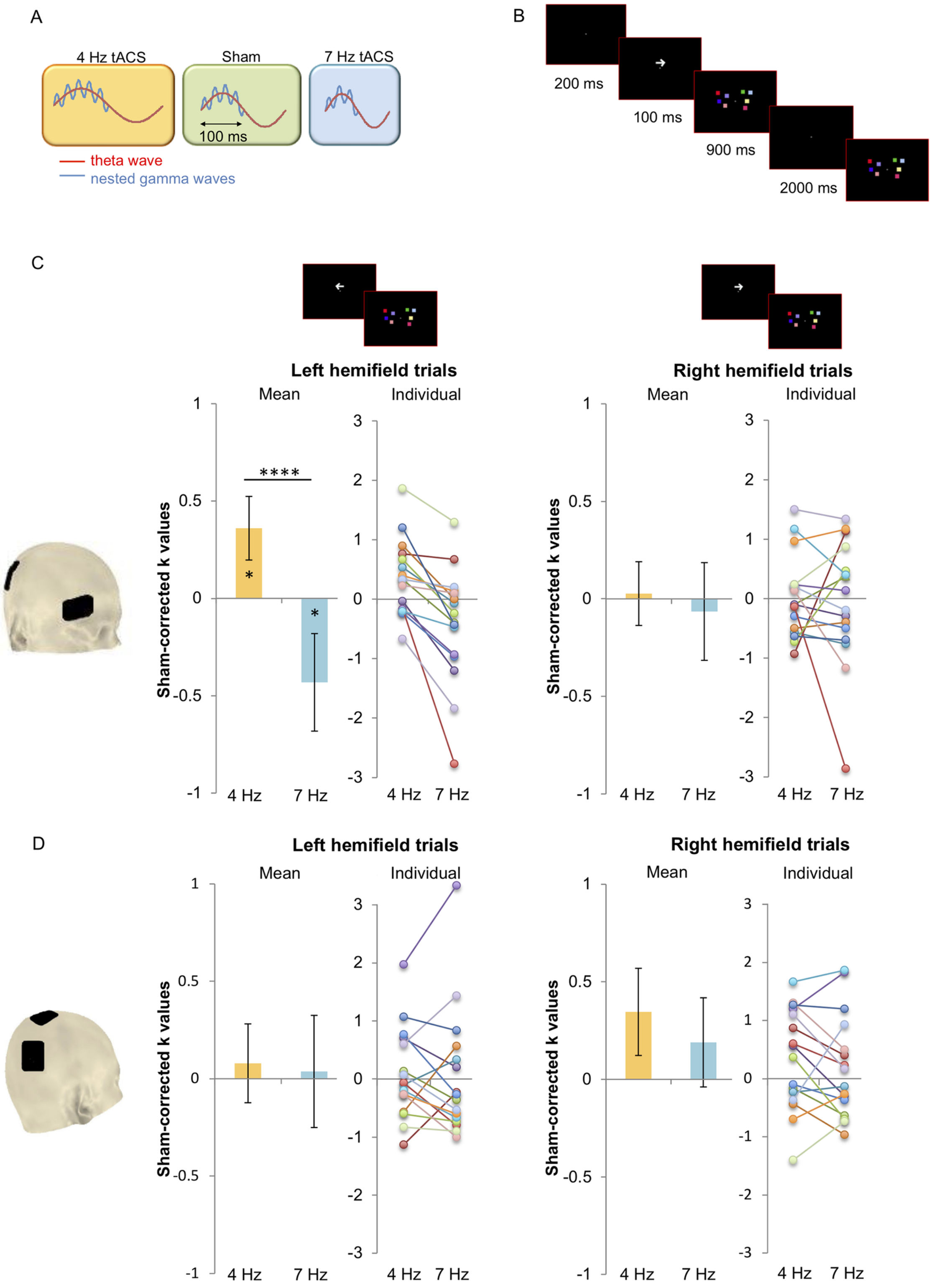

Fig 1. (A) Theta–gamma phase coupling theory. The maximum number of items stored in WM is thought to be a function of the number of gamma cycles nested into a theta wave [1,2]. We tested this theory by applying slower (4 Hz) and faster (7 Hz) theta frequency tACS, aiming at modulating the speed of theta cycles (as per the entrainment hypothesis; see [10,12,13,19]) to allow higher/lower numbers of gamma cycles nested within a theta phase. Four-hertz tACS (yellow panel) should slow down theta oscillations, allowing more gamma cycles to nest within a theta cycle, relative to sham (green panel), enhancing WM capacity. Seven-

hertz tACS (blue panel) should speed up theta oscillations, allowing fewer gamma cycles to nest within a theta cycle, relative to sham (green panel), worsening WM capacity. **(B) Visual delayed match to sample task**. Two arrays of coloured squares were situated on either side of a white fixation cross in the centre of a black screen. The number of squares in each array (memory load) was 4, 5, or 6, with 20 trials presented for each load. The task started with a fixation cross on the screen. Prior to presentation of the arrays, an arrow appeared on the screen (200 ms) to indicate which of the two upcoming arrays (left or right) needed to be memorised. The two arrays then appeared on the screen (100 ms), followed by a retention interval (900 ms), again followed by two arrays (left and right; 2,000 ms). Participants had to indicate whether the array in the cued hemifield had changed. **(C) (Experimental Montage) and (D) (Control Montage): results** (underlying data can be found at: https://osf.io/rm6qp/). K-values for each combination of load and hemisphere for each condition per participant were calculated with the formula: (hit rate − false alarms) * set size [20] (see Data analysis). Leftmost graphs depict mean and individual K-values obtained for trials presented on the left hemifield for each active stimulation condition after sham correction (Sham-corrected 4 Hz, Sham-corrected 7 Hz). Rightmost graphs depict mean and individual K-values obtained for trials presented on the right hemifield for each active stimulation condition after sham correction (Sham-corrected 4 Hz, Sham-corrected 7 Hz). Significant differences between conditions were observed for the Experimental Montage (C) but not for the Control Montage (D) and only for stimuli presented to the left hemifield (i.e., contralateral to the stimulated parietal site). For non-sham-corrected K and accuracy data, see S1A and S1B Fig (S1A and S1B Table), respectively (underlying data can be found at: https://osf.io/rm6qp/). $^{*}p < 0.05$; $^{****}p < 0.0001$. Error bars depict standard error of the mean. tACS, transcranial alternating current stimulation; WM, working memory.

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Results

Two groups of 16 participants were each assigned to two different electrode montages. In both montages, an electrode was placed over the same right parietal region in order to stimulate the parietal area of the frontoparietal WM network, known to be relevant in visuospatial WM tasks [14]. This electrode was paired with either a return electrode over the vertex (Control Montage) or over the right supraorbital region (Experimental Montage). A few potential issues were anticipated with the use of the Control Montage for our paradigm to be effective: (a) the reduced distance between the two electrodes in the Control Montage, which might result in a significant proportion of current being shunted over the skin [15], rendering the stimulation less effective; (b) the spread of current across both hemispheres, due to the vertex electrode sitting centrally and therefore reducing the expected lateralised impact of stimulation on WM performance [3,7,8,9]; (c) the different orientation of current flow relative to the neurons' orientation across the two montages, specifically due to the differential position of the return electrode, which may play a relevant role in the stimulation efficacy [16,17]; and (d) the Control Montage being less effective on the target brain area intraparietal sulcus (IPS) (see [18]). The Experimental Montage used was designed to overcome these potential confounds.

Participants in each montage group underwent active stimulation (at 4 and 7 Hz) and sham stimulation while performing a visuospatial delayed match to sample task [3,7,8,9]. The visuospatial WM task involved remembering an array of four to six coloured squares that was briefly presented to either the left or right visual hemifield (i.e., contralateral or ipsilateral to the stimulated hemisphere, respectively) for a short period of time and then assessing whether it was the same or different from a subsequently presented array (see Fig 1B for details on the task and stimuli example).

WM capacity across different memory loads was measured using a K-value, which is a standardised measure estimating how many items can be stored in WM (e.g., [3,7,8,9]). In addition, in order to make the results more comparable with other studies not using K as an estimate of memory capacity, the percentage of correct responses (accuracy) was also calculated. Finally, in order to reduce variability induced by the control condition sham (as the between-group factor), data were sham-normalised (for a non-sham-corrected data analysis, see S1 Fig).

A mixed factorial ANOVA with the between-factor Montage (Experimental Montage and Control Montage) and within-factors Condition (Sham-corrected 4 Hz, Sham-corrected 7 Hz) × Load (4, 5, and 6 items) × Hemifield (left and right) was carried out on the K-values (see Fig 1C and 1D, and Data analysis) and accuracy (see S1 Fig).

Results showed a main effect of Condition (K: F(1,30) = 5.90, $p = 0.021$, $\eta^2 = 0.16$; accuracy: F(1,30) = 6.39, $p = 0.017$, $\eta^2 = 0.18$), suggesting that stimulating at 4 Hz and 7 Hz relative to Sham had a differential impact on WM capacity. Importantly, a Condition × Hemifield × Montage interaction (K: F(2,60) = 5.79, $p = 0.022$, $\eta^2 = 0.16$; accuracy: F(2,60) = 5.25, $p = 0.029$; $\eta^2 = 0.15$) showed that the two montages modulated performance differently depending on stimulation Condition and Hemifield. Subsequent ANOVAs were therefore performed separately for each montage. In the Experimental Montage, we found a main effect of Condition (K: F(1,15) = 5.70, $p = 0.03$, $\eta^2 = 0.28$; accuracy: F(1,15) = 5.75, $p = 0.029$, $\eta^2 = 0.28$) and a significant interaction of Condition × Hemifield (K: F(1,15) = 9.53; $p = 0.008$; $\eta^2 = 0.39$; accuracy: F(1,15) = 7.46; $p = 0.015$; $\eta^2 = 0.33$), suggesting that the different stimulation conditions had a differential impact on left and right hemifields. Given the lateralised application of tACS (right parietal) and the contralateral parietal activation during visuospatial WM maintenance observed in previous work [3,7,8,9], a significant modulation of WM capacity was expected for items presented over the left (contralateral) but not the right (ipsilateral) hemifield. These trials were analysed separately in two further repeated measures ANOVAs (Condition × Load).

As expected, the analysis of the left hemifield trials showed a significant main effect of Condition (K: 4 Hz: 0.36 ± 0.016; 7 Hz: −0.43 ± 0.25; F(1,15) = 23.97; $p = 0.0002$; $\eta^2 = 0.61$; accuracy: 4 Hz: 3.54% ± 1.71%; 7 Hz: −3.44% ± 1.94%; F(1,15) = 45.53, $p = 0.000007$, $\eta^2 = 0.75$), while no main effect of Load nor interactions reached significance (K: all $p > 0.42$; accuracy: all $p > 0.78$). Moreover, one-sample *t* tests against 0 confirmed that 4-Hz tACS significantly enhanced K-values (and accuracy) relative to sham (K: $t(15) = 2.28$; $p = 0.019$, one-tailed; Cohen's $d = 0.57$; accuracy: $t(15) = 2.13$; $p = 0.024$, one-tailed; Cohen's $d = 0.53$), while 7-Hz tACS significantly reduced K-values (and accuracy) relative to sham ($t(15) = -1.78$; $p = 0.047$, one-tailed; Cohen's $d = 0.44$; $t(15) = -1.83$; accuracy: $p = 0.046$, one-tailed; Cohen's $d = 0.46$) (Fig 1C leftmost graphs for mean and individual data and S1 Fig).

As expected, analysis of the right hemifield trials showed no significant effect of Condition (K: F(1,15) = 0.12; $\eta^2 = 0.008$; $p = 0.73$; accuracy: F(1,15) = 0.002; $p = 0.97$; $\eta^2 = 0.0001$), as well as no significant effect of Load or interactions (all $p > 0.19$) (Fig 1C, rightmost graphs and S1 Fig). Finally, the same analysis performed on the Control Montage showed no main effects or interactions reaching significance (all $p > 0.23$) (Fig 1D, S1A and S1B Fig).

In order to clarify the contribution of the electrode configuration on the observed effects, we calculated electric field distribution for both montages based on a realistic head model [21]. Results of this analysis suggest that stimulation in the Control Montage (P4-Cz) led to more superior parietal stimulation and more left parietal stimulation, relative to our Experimental Montage (P4-supraorbital), in which participants received stronger stimulation exactly at around the right IPS [18] that then spread throughout the right hemisphere but was confined within it (Fig 2).

Discussion

In this study, we show for the first time that by applying tACS over the parietal cortex at a slow theta frequency (4 Hz), we were able to boost visuospatial WM capacity. On the other hand, stimulating at a faster theta frequency (7 Hz) reduced visuospatial WM capacity. Modulation of WM capacity was selective for stimuli presented in the hemifield contralateral to the stimulated hemisphere, in line with previous reports [3,7,8,9] showing a lateralised parietal activation during stimulus retention, which correlates in turn with individual WM capacity. Moreover, these effects were observed only when the right parietal electrode was paired with the right supraorbital return electrode (Experimental Montage) but not with the Cz return

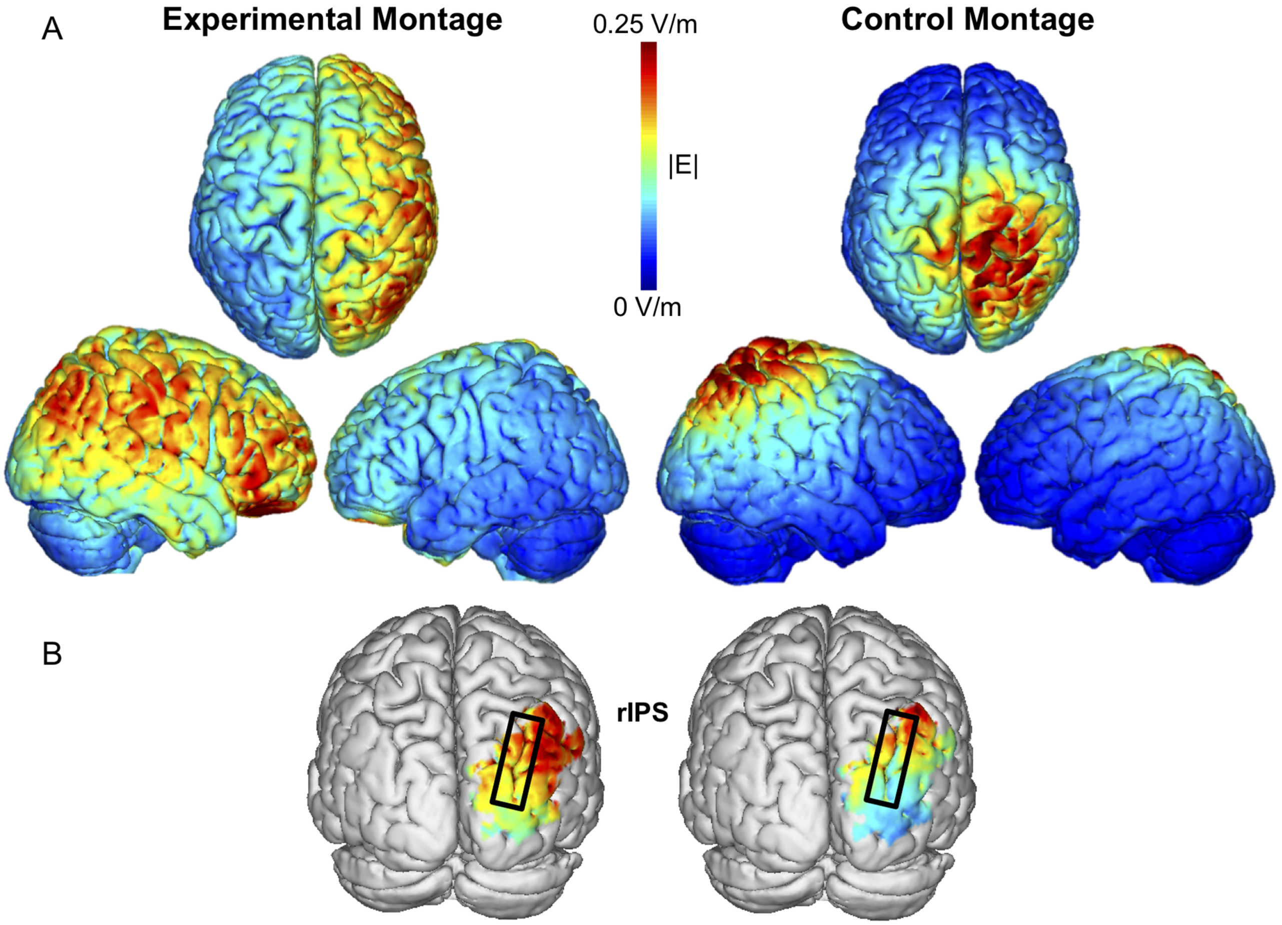

Fig 2. (A) Electric field distribution calculation (NIC 2.0 Software: http://www.neuroelectrics.com/products/software/nic2/) for Experimental (left) and Control (right) electrode montages. The Experimental Montage (P4-supraorbital) shows (i) a more right-lateralised field distribution with (ii) maximum current over more posterior parietal areas relative to the control montage (P4-Cz). The Control Montage shows (i) some left-lateralised field distribution with (ii) maximum current over right superior parietal areas, thus more anterior to P4. These differences in electric field distribution might be responsible for the significantly different impact obtained across the two montages and may possibly explain the null effects obtained using the Control Montage. **(B) Masked display with highlights of right IPS electric field distribution calculation** (NIC 2.0 Software: http://www.neuroelectrics.com/products/software/nic2/), relative to the Experimental (left) and Control Montages (right). Here, it can be more closely appreciated how the Experimental Montage induced a maximum current over IPS, relative to the Control Montage. IPS, intraparietal sulcus; NIC, Neuroelectrics Instrument Controller; rIPS, right intraparietal sulcus.

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electrode (Control Montage). Importantly, these results lend behavioural support to the seminal work by Lisman and Idiart [1], suggesting that a slower theta frequency would code for a higher number of gamma cycles, leading to enhanced WM capacity, whereas a faster theta frequency codes for a smaller number of gamma cycles, leading to reduced WM capacity.

On the nature of tACS effects

The effects we obtained were in line with the expected empirical results. Theoretically, the difference between cycle length of 4 Hz (250 ms) and 7 Hz (143 ms) would be around 107 ms and would therefore allow for at least two additional gamma cycles to be nested into a theta cycle. Based on this evaluation, a difference of 2 items between stimulation conditions could be expected when stimulating at 4 Hz relative to 7 Hz. However, we note that the modulation of the K-values obtained here at 4 Hz and 7 Hz does not perfectly match the impact that would be expected from theory (see Fig 1A). Specifically, we found a total difference across conditions of about 0.8 items, which in terms of accuracy corresponds to about a 7% difference between 4 Hz and 7 Hz stimulation. This difference between the potential maximum impact of stimulation and the observed effect can be explained by the fact that tACS delivers very weak currents that might only partially drive the endogenous ongoing oscillatory activity. There is also recent evidence showing how the impact of tACS on oscillatory activity is strictly dependent on the state of the brain during stimulation [22]. This implies that intervening factors can also induce subtle modulation of tACS effects, rendering the stimulation less effective. For example, in our experimental manipulation, tACS was not delivered in phase with the beginning of the 900-ms retention period shown to be more strictly related to theta synchronization [2]. This might account for some trial-by-trial variability in the impact of theta tACS for maximising cross-frequency coupling in the relevant theta phase (i.e., stimulation may have been more effective in those trials in which tACS was in phase with the onset of the retention interval than in other trials that were less in phase or even in counterphase with the onset of the retention period). Moreover, one would only expect a full 2-item difference if each participant responded perfectly. Yet, individual differences might significantly interact with the way the method optimally works, and therefore one would also expect overall smaller mean values. However, despite such potential intervening factors, tACS still significantly impacted current results in the predicted direction.

WM capacity and theta oscillatory peak: A stable, trait-like phenomenon, with functionally significant variations around the mean

WM capacity shows variability both between and within participants. Between-participants variability in WM capacity has been documented. For example, it has been shown that WM variance can be explained by the theta:gamma frequency ratio [23], and even within participants, trial-by-trial variability can be observed [24], with the same items being sometimes retained and sometimes not. We speculate that this variability might be reflected in slight variations in oscillatory activity related to this function. Indeed, recent reports support the functional relevance of the between and within trial-by-trial variability in frequency speed in different domains, from visual processing [11,25] to multisensory binding [12] and pain perception [26], thus rather discarding the notion of this being sheer noise in oscillatory fluctuations. Although not directly related to our experimental paradigm or specific oscillatory frequency, these findings provide a more general framework in support and physiological backup to the behavioural results we present here, suggesting that tACS can effectively shift individual trait-like behaviour associated with oscillatory activity in desired directions. In the context of WM capacity, the model of Lisman and Idiart [1] very well matches the theoretical framework of inter- and intraindividual oscillatory variability determining WM capacity, and we have indeed tested this in the current study. Therefore, while WM capacity is a trait-like ability, possibly centred around a person's individual theta frequency peak, this trait-like dimension may be prone to slight but functionally significant fluctuations around the mean, which could be best explained by the trial-by-trial variability in the theta frequency peak, accounting for the trial-by-trial ability to correctly encode information in WM.

Interindividual variability in theta frequency may impact the way tACS modulates WM capacity

Individual theta frequency may vary across participants in the full range between 3 and 8 Hz, thus even beyond the range of 4–7 Hz that we have considered. This might in principle explain why not all of our participants showed a consistent effect of enhanced or reduced WM capacity relative to their sham condition. According to our hypothesis, one would expect that 4-Hz tACS would always improve performance relative to sham and 7-Hz tACS would always reduce performance relative to sham. While at first sight, this might seem to be the case, this conclusion would be based on the misleading assumption that, at the individual level, sham stimulation would necessarily sit in the middle as if it corresponded to a 5.5-Hz stimulation. However, as per definition, individual theta frequency may vary within an even wider range than the one we have defined here, conventionally and conveniently, but arbitrarily, of 4 and 7 Hz. Indeed, while literature generally refers to theta as an oscillatory activity in a range between 4 and 7 Hz, it has been reported that both slow theta 3-Hz oscillations and fast theta 8-Hz oscillations can be associated with memory performance [27]. Therefore, 4- and 7-Hz stimulations do not necessarily sit on the lower and upper boundaries of the individual theta frequency. In turn, this may explain in principle why some participants do not show positive Sham-corrected values for 4-Hz stimulation with negative differential scores for 7-Hz stimulation at the same time, or being close to 0 in other cases. Crucially, this perspective would also explain why, in all cases, 4-Hz relative to 7-Hz stimulation always resulted in a better WM performance.

At an additional level of analysis, the shape of the individual theta peaks may also vary quite substantially from sharp to broad across individuals, which might in turn determine the net effect size of our data. Indeed, such characteristics may well interact in the way participants respond to the tACS interventions, such that participants with broadband theta might be more susceptible to tACS interference than those with sharp peaks. While we cannot provide a more detailed and conclusive demonstration of this mechanism here, these are all relevant points that future research needs to address. However, the current study already provides a fundamental step forward in the understanding of the mechanisms underlying spatial WM processing, showing the behavioural impact of tACS modulation of WM capacity closely following a theoretical model of WM capacity on the one hand and interventional impact of tACS on the other hand.

The theta–gamma phase coupling hypothesis

It might be argued that a way to alternatively demonstrate the impact of tACS on WM capacity would instead be to modulate gamma frequency. So, in principle, stimulating at faster gamma frequency might enhance WM capacity, while stimulating at slower gamma frequency would instead reduce WM capacity. However, the theta–gamma coupling framework of WM capacity does not assume any changes in gamma frequency and, indeed, there is clear evidence against this. For instance, Axmacher and colleagues [2] showed that increasing WM load leads to a reduction in theta frequency, whereas gamma frequency is not significantly slowed down. Also, the theoretical framework assumes that single memory item representations would be reflected by activity in local gamma networks oscillating at exactly this gamma frequency. The single representations themselves would not change, therefore gamma frequency should not either. In support of this notion, there is evidence that locally generated gamma would not even change frequency if the network size were increased [28].

Electric field modelling reconstruction: A role for frontal versus parietal stimulation?

Based on the electric field modelling reconstruction, the Experimental Montage shows its maxima over IPS, exactly underneath the stimulation electrode, as one would expect. The control condition shows instead a maximum more anterior, off the stimulation electrode, with a clearly less lateralised distribution of the electric field, which together could explain why, within our paradigm, there was no significant modulation of WM capacity in the Control Montage.

When looking at the Experimental Montage electric field distribution, this is clearly lateralised with maxima over the IPS and spread more widely through the right hemisphere, including frontal areas. According to this picture, it could be argued that the actual significant impact of the Experimental Montage might be due (i) to a more effective tACS of the right prefrontal cortex via the supraorbital electrode of the Experimental Montage or (ii) to a more effective activation of the frontoparietal network instead and not the P4 stimulation site per se, common to both montages, or even (iii) subcortical activations. Although we cannot completely rule out these alternative hypotheses, we argue that these are very unlikely explanations of current results. First, the right supraorbital electrode is not the optimal position for modulating frontal theta oscillatory activity (see previous tACS work targeting the frontoparietal network, with the frontal sensor sitting more posteriorly, e.g., over FCz or laterally over F3 and F4 [6,29,30]). Moreover, tACS montages testing the relative impact of frontal and parietal areas have shown a selective modulation of WM by parietal but not frontal stimulation [29,30]. Therefore, our Experimental Montage has likely not desynchronised frontal and parietal areas that were actively involved in the WM task but instead optimised a lateralised stimulation of one of the crucial nodes (right parietal area in this case) leading to current lateralised effects. Furthermore, if any of the effects observed could be ascribed to activation of frontal or even subcortical activations, it is unlikely to carry lateralised effects, which should instead be driven more specifically by parietal activations. Importantly, the choice of the parietal area was strongly inspired by relevant empirical work [3,7,8,9]. These studies showed that the spatial component is a relevant one in our experimental design that actively calls into play parietal rather than frontal activations. Indeed, both theta oscillations [3] and event related potential (ERP) components associated with spatial WM capacity [7,8,9], in the very same paradigm we have used here, are systematically localised contralateral to the items to be remembered and are crucially over posterior areas, the very same we have stimulated in our study. Instead, no such modulation of theta oscillations [3] or ERP [7,8,9] over prefrontal areas has been found whatsoever. Indeed, we are considering here a specific visuospatial cued memory component that strongly relies on the visuospatial components, for which parietal areas are primarily involved. It is very unlikely that such lateralised effects observed here and replicated numerous times (see, e.g., [31,32]) in different visuospatial WM experimental paradigms (see, e.g., [33,34]) may be driven by frontal activations. Therefore, based on this evidence, we explicitly expected the effects to be mainly driven by parietal stimulation and to be lateralised.

Cortical versus retinal effects

One could argue that the effect we observed could be the result of retinal activation [16,35] due to supraorbital electrode stimulation. Several arguments, however, discount this alternative hypothesis. First of all, none of the participants saw any phosphenes during the experiment. Indeed, reports of retinal phosphene for stimulation frequency within the theta band are very rare (see [36]). Those few participants who saw phosphenes at the beginning ($n = 3$) were stimulated during the experiment at a tACS intensity not inducing any phosphene sensation. Yet, if one has to consider the potential impact of some residual retinal phosphenes perception over the WM capacity using the current paradigm, a different pattern of results would be expected than the one we currently observe. Specifically, we would expect any effect to be essentially ipsilateral to the stimulation site rather than contralateral, as observed here, instead. While we do observe a lateralised effect of stimulation on WM capacity, no main effect of hemifield could be detected, but only an interaction between hemifield and the specific frequency of stimulation. Such effects were observed for the contralateral rather than ipsilateral hemifield to the stimulated hemisphere and retina and are thus unlikely to be explained by retinal activation.

Finally, if phosphenes were not perceived, it might still be argued that a subthreshold impact of tACS on retinal activity might be induced, which may in turn indirectly induce cortical entrainment in corresponding visual areas. If this were to be the case, we would still argue that the activation of the ipsilateral retina would project onto both hemispheres, thus inducing a bilateral entrainment, which is not compatible with the lateralised effect we observed in the current study. Therefore, we have good reasons to believe that any of the effects we observed were genuinely cerebral in nature.

Conclusions

To conclude, we found that, depending on electrode configuration, 4-Hz tACS enhanced visuospatial WM capacity, while 7-Hz tACS reduced visuospatial WM capacity compared to sham stimulation. As a result of the hemifield specificity effect, each participant served as their own internal control, depending on the hemifield being tested relative to the hemisphere being stimulated, with the effects being found for items presented to the hemifield contralateral to the stimulation site only. These results are also supported by recent reports in monkeys [37] performing a similar change detection task and showing an independence of the two hemispheres for visual WM function.

The findings of this study are in line with the theta–gamma phase coupling theory of WM capacity [1]. While direct electrophysiological evidence supporting our conclusions is still lacking due to the technical challenge of combining online tACS and EEG (see [6,10]), here we provide relevant behavioural evidence that slow theta tACS enhances visuospatial WM capacity and fast theta tACS reduces visuospatial WM capacity, in line with the idea that slow and fast theta tACS might induce slower and faster theta oscillations, respectively (see [10,11,12,13]). These findings may provide the basis for potential therapeutic interventions aimed at enhancing poor memory capacity in the ageing population and to ameliorate memory-related neuropathologies in clinical settings.

Materials and methods

Ethics statement

The study has been approved by the University of Essex Ethics Committee (VR1301) and conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent has been obtained from the participants before taking part in the study.

Participants

Based on power analysis (conducted on [6], actual effect size, f = 0.47), with a conservative estimated effect size f = 0.25, alpha = 0.05, and 80% power, a total sample size of 28 participants (14 per group) is suggested. Therefore, in a between-subjects design, we assigned 16 participants (7 female) to the Experimental Montage and 16 (9 female) to the Control Montage. All participants were adults (18 years or older) with mean ages of 28.3 years (±7.6) and 22.8 years (±5.2), respectively. Participants completed a safety screening questionnaire before taking part in each session of the study.

Design

A repeated measures design was used for stimulation conditions and memory load. There were two active stimulation conditions (4- and 7-Hz tACS) and one control condition (sham). These stimulation conditions took place during three separate sessions that occurred on separate days, and the order was counterbalanced across participants. At least 24 hours passed between different sessions. A single-blind design was used, with participants unaware that different stimulation protocols were being used for each session (and unaware that one session consisted of sham stimulation).

Stimulation protocol

Experimental Montage. Two 5 × 7 cm electrodes were placed over the scalp using saline-soaked sponges. Prior to electrode placement, skin was prepped with alcohol wipes to improve conductivity. One electrode was placed over the right parietal cortex (corresponding to P4, according to the 10–20 system) and the other was placed on the forehead above the right eyebrow (supraorbital). Maximum stimulation intensity was initially set to 1,500 μA peak-to-peak amplitude and was delivered using a Magstim DC-Stimulator Plus device.

During the first session, stimulation at 1,500 μA was tested to see if retinal phosphenes occurred. If none occurred, this level of stimulation was used. If retinal phosphenes occurred at this level, the individual threshold for retinal phosphenes was determined for that participant. This was done by starting at 1,000 μA and increasing in increments of 25 μA or decreasing in increments of 100 μA and then back up in increments of 25 μA until the highest level that did not produce retinal phosphenes was found. The average level of stimulation was 1,238 μA (±298 μA). For the second and third sessions, the voltage determined in the first session was used. In three cases, retinal phosphenes were reported at this level during the second or third session and the voltage was therefore reduced for the remaining sessions for those three participants (using the method outlined above to determine the appropriate level). In these cases, this resulted in differences of −50 μA, −650 μA, and −400 μA for the 7-Hz session relative to the 4-Hz session. No other participants reported retinal phosphenes at the threshold determined during the first session.

All conditions consisted of a ramping up stage at the beginning of the stimulation. For the 4-Hz condition, this lasted for 120 cycles; for the 7-Hz condition, this lasted for 210 cycles; and for the sham condition, this lasted for 165 cycles. For the 4- and 7-Hz conditions, this was followed by stimulation at the predetermined threshold or at 1,500 μA; for the sham condition, the ramping up period was followed by stimulation for 165 cycles and then a fade-out period that lasted 165 cycles. The brief stimulation period in the sham condition was carried out to mimic any sensations that may have been felt during the active stimulation conditions. This also allowed the retinal phosphene threshold to be determined when the first session contained sham stimulation. During the ramping-up, stimulation, and fade-out periods in the sham condition, the frequency of stimulation was at 5.5 Hz. This frequency was chosen as a neutral frequency relative to the two active conditions. During the sham session, participants performed the task only after the fade-out period. Throughout all stimulation sessions, impedance levels were kept below 5 kΩ.

Control Montage. Two 5 × 7 cm electrodes were placed over the scalp using saline-soaked sponges. Prior to electrode placement, skin was prepped with alcohol wipes to improve conductivity. One electrode was placed over the right parietal cortex (corresponding to P4, according to the 10–20 system) and the other was placed on the vertex (corresponding to Cz, according to the 10–20 system). Stimulation was delivered using a Magstim DC-Stimulator

Plus device. Stimulation was delivered at 1,500 μA. None of the participants reported retinal phosphenes at this level during any session.

All conditions consisted of a ramping up stage at the beginning of the stimulation, and the procedure for this was the same as with the Experimental Montage. As with the Experimental Montage, the frequency of stimulation during the ramping up and fade-out periods in the sham condition was 5.5 Hz and the total duration of stimulation was 495 cycles, inclusive of the ramping up and down periods. Throughout all stimulation sessions, impedance levels were kept below 5 kΩ.

Materials

A variation of the visual delayed match to sample task based on Vogel and Machizawa [8] was used. Two arrays of coloured squares were situated on either side of a white fixation cross in the centre of a black screen. The number of squares in each array (the memory load) was 4, 5, or 6. The left and right arrays were always different from each other in any given trial; however, the number of squares in each trial was always the same for the left and right arrays.

Behavioural task. The task started with a fixation cross on the screen. Prior to presentation of the arrays, an arrow briefly appeared on the screen (200 ms) to indicate which of the two upcoming arrays (left or right) needed to be memorised. The two arrays (one on the left and one on the right of the fixation cross) then appeared on the screen for 100 ms. This was followed by a retention interval of 900 ms, during which there was a fixation cross on the screen. After the retention interval, two arrays appeared on the screen for 2,000 ms (again, there was one array on either side of a fixation cross). Participants had to indicate whether this array on the previously indicated side was the same as or different than the array on that side presented previously during that trial. This was done by pressing either the left or right button of the mouse (left to indicate that the arrays were the same and right to indicate that they were different). The order of trials within each block was randomised by the computer program.

There were 6 blocks of 20 trials presented during each stimulation session. Each block contained trials of one load only (4, 5, or 6). For each session, the blocks were chosen at random from a set of 9 blocks (3 for each load) with the following restrictions: exactly 2 blocks of each load were to be used and no block was to be repeated during any given session. These 6 blocks were presented in a randomised order. Each block contained 5 left-mismatched, 5 left-matched, 5 right-mismatched, and 5 right-matched trials.

Experiment procedure. After the setup of the electrodes, participants were presented with written instructions. Participants were instructed to use only one hand to make their responses and were given the opportunity to clarify the instructions if needed. The lights were turned off and participants carried out a practice block of trials. This practice block used load 3 stimuli to prevent load-specific practice effects from affecting subsequent performance.

After the practice block had finished, additional saline solution was added to the sponges and stimulation was started. Participants were then instructed to start the first block when ready. After each experimental block, participants pressed the enter button to continue to the next block. Each block lasted approximately 2 minutes. Therefore, the total task time during stimulation lasted approximately 12 minutes. Participants were given monetary compensation or course credits for their participation after each of the three sessions and were debriefed after the final session and invited to ask any questions.

Data analysis. Participant responses were preprocessed before undergoing statistical analysis. A mixed repeated measures design was used with the between-factor Montage and the within-factors Load, Hemifield, and Condition (Stimulation Frequency).

Because there were three different load conditions (4, 5, and 6), a simple analysis of the correct response was not optimal, and instead, a K-value was implemented as a dependent variable (in addition to accuracy). K is a standardised measure [20] of WM capacity that takes set size into account (e.g., [3,7,8,9,20,34,38,39,40]) and is defined as $K = S * (H - F)$, where H is the hit rate (i.e., the percentage of trials correctly recognised as the same or different), F is the false alarm rate (i.e., the percentage of trials erroneously judged as the same when they are different and vice versa), and S is the set size (i.e., the number of items presented in each trial, which could be either 4, 5, or 6 in the current task). K-values (and accuracy) were determined for each Montage, Condition, Load, and Hemifield combination.

The within-subjects design included two active (4- and 7-Hz) and one sham stimulation condition. The two active conditions were normalised to the sham condition (4 Hz minus sham and 7 Hz minus sham) for each montage, load, and hemifield (for completeness, non-sham-corrected data are reported in S1 Fig). A mixed repeated measure ANOVA was conducted with the between-subjects factor Montage (Experimental and Control Montage) and the within-subjects factors Condition (Sham-corrected 4 Hz, Sham-corrected 7 Hz), Load (4, 5, and 6), and Hemifield (left and right). When interactions reached significance, subsequent ANOVAs were performed separately for Montage and Hemifield, followed up by direct contrast paired *t* test between Sham-corrected 4 and 7 Hz, when appropriate. Sham-corrected 4 and 7 Hz were also submitted to a one-sample *t* test against 0 to test whether they significantly differed from sham. One-tailed *t* tests were used as we anticipated directionality of the effects for the 4- and 7-Hz stimulation relative to each other and to sham, based on the theoretical model and empirical evidence upon which the experimental design was conceived.

Neuroelectrics Instrument Controller (NIC) 2.0 software was used to calculate electric field distribution. The software is based on the approach developed and described by Miranda and coworkers [21]: In NIC 2.0, the electric field E at the interface between cerebrospinal fluid and grey matter was estimated in an MRI-based realistic head model [21]. This head model was built from a Colin 27 template obtained from BrainWeb [21]. In this model, different kinds of tissue were modelled as homogeneous and isotrophic media. Electrical conductivity was estimated with 0.33 S/m for the scalp and grey matter, 0.15 S/m for white matter, 0.008 S/m for the skull, and 1.79 S/m for cerebrospinal fluid. The sponge electrodes were estimated with a conductivity of 2 S/m [21]. At the interface between cerebrospinal fluid and grey matter, a finite element mesh was created using Comsol software. Based on Comsol software, Laplace's equation for the electric potential was used. Then, the electric field at all nodes of the mesh was estimated by using the gradient of the electric potential [21]. In Fig 2, the magnitude of the electric field $|E|$ is displayed separately for the Experimental Montage (P4-supraorbital) and the Control Montage (P4-Cz).

Supporting information

S1 Fig. Analysis performed on non-sham-corrected (A) K-values and (B) accuracy for the Experimental and Control Montages: Results (underlying data can be found at: https://osf.io/rm6qp/). Leftmost graphs depict mean K-values and accuracy obtained for trials presented on the left hemifield for each active (4- and 7-Hz) and sham condition, while rightmost graphs depict mean K-values and accuracy obtained for trials presented on the right hemifield for each active and sham condition. Significant differences between stimulation conditions were observed for the Experimental but not for the Control Montage and only for stimuli presented to the left hemifield, leftmost graph (i.e., contralateral to the stimulated parietal site). $^{*}p < 0.05$; $^{****}p < 0.0001$. Error bars depict standard error of the mean. Unlike the sham-corrected analysis presented in the main text, this data analysis does not factor out the variability induced by

including the control factor "sham" in the between-group design, resulting in weaker between-group effects. The mixed factorial ANOVA with the between-factors Montage (Experimental versus Control) and within-factor Condition (4 Hz, 7 Hz, and Sham) × Load (4, 5, and 6 items) × Hemifield (left and right) carried out on the K-values and accuracy showed an effect of Load (K: (F2,60) = 26.4; $p < 0.00000001$; $\eta^2 = 0.47$; accuracy: F(2,60) = 82.848; $p < 0.00000000001$; $\eta^2 = 0.734$), confirming that the task is generally more challenging for higher than lower loads. A Condition × Hemifield × Montage marginal interaction (K: (F2,60) = 2.72; $p = 0.074$; $\eta^2 = 0.083$; accuracy: F(2,60) = 2.47; $p = 0.093$; $\eta^2 = 0.076$) suggests that the two montages had a different impact on performance, depending on stimulation Condition and Hemifield. To further ascertain the nature of the impact of montage on frequency-specific effects, the same ANOVA was performed separately for each group. In the Experimental Montage, we confirmed an effect of Load (K: F(2,30) = 19.12; $p < 0.000001$; $\eta^2 = 0.56$; accuracy: F(2,30) = 52.55; $p < 0.00000001$; $\eta^2 = 0.78$. In addition, we found a main effect of Condition (K: F(2,30) = 3.29; $p = 0.05$; $\eta^2 = 0.18$; accuracy: F(2,30) = 3.69; $p = 0.036$; $\eta^2 = 0.20$) and a significant interaction Condition × Hemifield (K: F(2,30) = 3.70; $p = 0.037$; $\eta^2 = 0.198$; accuracy: F(2,30) = 3.61; $p = 0.039$; $\eta^2 = 0.194$), suggesting that the different stimulation conditions had a differential impact on left and right hemifields. These trials were analysed separately in two further repeated measures ANOVAs (Condition × Load). As expected, the analysis of the left hemifield trials showed again an effect of Load (K: F(2,30) = 5.49; $p < 0.01$; $\eta^2 = 0.36$; accuracy: F(2,30) = 20.42; $p < 0.00000001$; $\eta^2 = 0.58$) and a significant main effect of stimulation condition (K: F (2,20) = 8.57; $p = 0.0011$; $\eta^2 = 0.364$; accuracy: F(2,30) = 9.94; $p < 0.001$; $\eta^2 = 0.40$), while no significant interaction between Load and Condition was observed (K: F(4,60) = 0.60; $p = 0.66$; $\eta^2 = 0.038$; accuracy: F(4,60) = 0.185; $p = 0.94$; $\eta^2 = 0.012$). Subsequent *t* tests revealed 4-Hz stimulation to induce higher K-values and accuracy compared to sham condition (K: $t(15) = 2.28$; $p = 0.019$, one-tailed; Cohen's $d = 0.57$; accuracy: $t(15) = 2.13$; $p = 0.024$; Cohen's $d = 0.53$); 7-Hz stimulation to induce lower K-values and accuracy compared to sham condition (K: $t(15) = 1.79$; $p = 0.047$, one-tailed; Cohen's $d = 0.45$; accuracy: $t(15) = 1.83$; $p = 0.043$, one-tailed; Cohen's $d = 0.46$); and 4-Hz stimulation to induce higher K-value and accuracy compared to 7-Hz stimulation condition (K: $t(15) = 5.11$; $p = 0.000065$, one-tailed; Cohen's $d = 1.278$; accuracy: $t(15) = 6.75$; $p < 0.00001$; Cohen's $d = 1.69$) (leftmost graphs). Analysis of the right hemifield trials showed only a main effect of Load (K: F(2,30) = 12.41; $p < 0.0001$; $\eta^2 = 0.45$; accuracy: F(2,30) = 37.22; $p < 0.00000001$; $\eta^2 = 0.71$) but no other main effects or interactions (all $p > 0.21$) (rightmost graphs). The same analysis performed on the Control Montage showed only an effect of Load (K: F(2,30) = 9.39; $p = 0.00068$; $\eta^2 = 0.385$; accuracy: F(2,30) = 33.40; $p < 0.00000001$; $\eta^2 = 0.69$), with no other main effects or interactions reaching significance (all $p > 20$).
(TIFF)

S1 Table. (A) K-values and (B) accuracy values (%) for each montage (Experimental and Control Montages), stimulation condition (4 Hz, 7 Hz, and Sham), Hemifield (left and right), and Load (4, 5, and 6 items) (underlying data can be found at https://osf.io/rm6qp).
(XLSX)

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