

# **Enhancing Neural Oscillations with Sawtooth Wave Transcranial Alternating Current Stimulation.**

Von der Fakultät für Medizin und Gesundheitswissenschaften der Carl von Ossietzky  
Universität Oldenburg zur Erlangung des Grades und Titels einer

Doctor of Philosophy (Dr. phil.)

angenommene Dissertation von

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**Tag der Disputation:** 19.09.2018

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(Published 2016, *Frontiers in Human Neuroscience*)

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## **Summary**

Non-invasive transcranial brain stimulation has huge potential as a research tool and a therapeutic intervention. Neural oscillations are fundamental to how the brain functions, and targeting neural oscillations using transcranial alternating current stimulation (tACS), is a promising research avenue. Until now, tACS has mostly been administered as an alternating sinusoidal wave. Evidence from animal models suggests that the gradient and direction of an electric current should be important factors for the subsequent neural firing rate. Despite modern tACS stimulators being able to deliver alternating current with any shape there has been no systematic exploration into the relative benefits of different waveforms.

Due to the variability across individuals and across time, as well as the complex feedback between stimulation and neuronal firing, monitoring of neural activity during stimulation is needed to fully understand its effects and to rapidly prototype new stimulation parameters. Stimulating with non-sinusoidal waveforms simplifies the problem of removing the large electrical artefact which is present when simultaneously stimulating and recording neural activity.

The two studies presented in this thesis use simultaneous sawtooth wave tACS and electroencephalography (EEG) to observe the effect of stimulation on neural oscillations using a novel artefact removal strategy. The first study compares the effect of various sawtooth waveforms on alpha oscillations and finds that positive ramp sawtooth, but not negative ramp sawtooth, significantly enhances alpha power during stimulation relative to sham. The second study investigates the effect of tACS on neural oscillations driven by visual flicker, the frequency and phase of which can be precisely controlled and targeted. A significant enhancement is found only when tACS and flicker are closely matched in frequency. Subsequent control experiments show no effect when the stimulating current is low or at nearby frequencies.

Taken together these two studies demonstrate that a significant modulation of neural oscillations can be observed in EEG during stimulation and this effect is dependent on the tACS waveform and frequency. This is an important first step in optimizing tACS for future research.

## **Zusammenfassung**

Nicht-invasive Gehirnstimulation hat großes Potential als Forschungsinstrument sowie für therapeutische Interventionen. Neuronale Oszillationen spielen eine grundlegende Rolle in der Gehirnfunktion und die Manipulation dieser mithilfe transkranieller Wechselstromstimulation (tACS) ist ein vielversprechender Forschungsweg. Bisher wurde tACS hauptsächlich als alternierende Sinuswelle appliziert. Belege aus Tiermodellen lassen vermuten, dass Stromgradient und -richtung entscheidende Einflussfaktoren auf die nachfolgende neuronale Feuerungsrate sind. Obwohl moderne tACS Stimulationsgeräte in der Lage sind, Wechselstrom beliebiger Form zu induzieren, wurden die relativen Vorteile verschiedener Wellenformen noch nicht systematisch untersucht.

Aufgrund individueller und zeitlicher Schwankungen sowie in Anbetracht des komplexen Zusammenhangs zwischen Stimulation und neuronaler Feuerungsrate ist es für ein vollständiges Verständnis der Effekte sowie für die schnelle Weiterentwicklung neuer Stimulationsparameter notwendig, die neuronale Aktivität während der Stimulation zu beobachten. Die Stimulation mit nicht-sinusförmigen Wellenformen vereinfacht das Problem, große elektrische Artefakte zu entfernen, die während des gleichzeitigen Stimulierens und Aufzeichnens neuronaler Aktivität auftreten.

Die beiden, in der vorliegenden Dissertation dargelegten Studien verwenden simultane Sägezahnwellen tACS und Elektroenzephalografie (EEG) zur Untersuchung der Stimulationseffekte auf neuronale Oszillationen mithilfe einer neuartigen Artefaktbeseitigungsstrategie. Die erste Studie vergleicht die Effekte verschiedener Sägezahnwellen auf Alpha-Oszillationen und führt zur Feststellung, dass positiv steigende Sägezahnwellen, jedoch nicht negativ steigende Sägezahnwellen, zu einem signifikanten Anstieg von Alpha-Power im Vergleich zu Placebostimulation führt. Die zweite Studie untersucht den Effekt von tACS auf neuronale Oszillationen, die durch visuelles Flimmern ausgelöst werden, dessen Frequenz und Phase präzise kontrolliert und manipuliert werden können. Ein signifikanter Anstieg ist ausschließlich zu finden, wenn die Frequenzen von tACS und Flimmern weitgehend deckungsgleich sind. Nachfolgende Kontrollexperimente zeigen keinen Effekt, wenn der zur Stimulation verwendete Strom niedrig oder in benachbarten Frequenzbereichen ist.

Zusammengefasst belegen die beiden Studien, dass eine signifikante Modulation neuronaler Oszillationen im EEG während der Stimulation beobachtbar ist und dass dieser Effekt von Wellenform und Frequenz der tACS abhängt. Dies ist ein wichtiger Schritt zur Optimierung der tACS für zukünftige Forschung.

# **Introduction**

## **2.1. Why stimulate the brain?**

Non-invasive transcranial brain stimulation has shown significant growth as a discipline in recent years and can be seen as a paradigm shift in both how the brain can be treated and as a research tool. Traditionally, treatment of the human brain has been limited to either pharmacology or surgery; while drugs offer the ability to modulate specific neurotransmitters or receptors, the spatial resolution is poor as the entire brain/body is affected; surgery on the other hand, offers high spatial precision but involves risks and is generally destructive and irreversible. The combination of invasive surgery and electrical stimulation, particularly deep-brain stimulation using implanted electrodes, has shown exciting results in recent years for conditions such as Parkinson's disease. However, this method still suffers from the drawback of requiring a dangerous and expensive surgical procedure as well as the limitation that it is difficult to prototype and develop new stimulation parameters as the risks of surgery has to be justified in each individual case. As such, transcranial stimulation seems to offer a potentially beneficial compromise as specific areas of the cortex can be targeted in a way that is non-permanent, generally well tolerated and safe for healthy volunteers with few side-effects (Poreisz et al., 2007; Rossi et al., 2009; Bikson et al., 2017)

## **2.2. Types of brain stimulation**

Historically the most common, and arguably the most clinically effective, form of transcranial stimulation has been electroconvulsive therapy (ECT) which involves large electrical currents applied to the head to induce a seizure, which in many cases provides relief from mental disorders such as treatment-resistant depression (Abrams, 2002). The mechanism by which ECT works is still poorly understood, despite being in use for over 70 years, but is thought to be a result of the induced seizure. Because the usage has been limited to clinical intervention and the mechanism of action is so different to other types of transcranial stimulation (due to the currents being much higher) I will not discuss ECT further, except in comparison to other brain stimulation types.

In the last 30 years two general types of transcranial stimulation have emerged and have entered widespread use: Transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (TES). TMS, as a method for stimulating the awake human brain, was pioneered in the mid-1980s by Anthony Barker (Barker, Jalinous, & Freeston, 1985). TMS involves a rapid change in current through a coil, or pair of coils, which generates a brief electric current in the cortex via induction. TES involves stimulating the brain via electrodes placed on the scalp.

A further subclass of transcranial brain stimulation which has attracted attention in recent years is focused transcranial ultrasound. Ultrasound can be focused (unlike electrical stimulation) which allows precise areas of the brain to be heated and destroyed (e.g. a tumour). In addition, some researchers have begun investigating the possibility of using focused ultrasound to modulate neural activity, this is however a very experimental method and beyond the focus of this thesis.

The resurgence of using weak electrical currents to modulate neural activity in humans began around the year 2000 following the work of Nitsche and Paulus who demonstrated that transcranial Direct Current Stimulation (tDCS) applied above the motor cortex could modulate the amplitude of motor evoked potentials (MEPs), with excitation following anodal stimulation, and inhibition following cathodal stimulation (Nitsche & Paulus, 2000).

Transcranial *direct* current stimulation (tDCS), a constant current with one anode and one cathode, has remained the most popular type of TES to date, partly due to it being the first variant of TES to be explored in detail, and partly due to its perceived simplicity. As such, the field of tDCS has attracted a lot of attention. Many clinical trials and neuro-psychological studies have been carried out, and a wide range of effects/benefits have been reported. However, in recent years the field has been increasingly aware that there are many failed replications and that in many cases single-session tDCS does not produce reliable cognitive effects (Horvath, Forte, & Carter, 2015). The over-hype, and subsequent backlash, seems to be the result of an overly simplistic understanding of how tDCS operates combined with publication bias and poorly controlled methodology (variations in montage/stimulation duration etc.). To counter this, many researchers have focused on the basic mechanisms and on modelling (in animals and computer simulations) exactly how TES achieves its effects as well as measurements in humans with intracranial recording electrodes to confirm, or refute, that current is reaching the cortex and affecting neuronal activity (e.g. Lafon et al., 2017; Huang et al., 2017).

### **2.3. Understanding how electrical currents affect the brain.**

Important advances in the understanding of the intensity and spatial distribution of current which reaches the cortex have been made with computational models. Such models typically use a structural Magnetic Resonance Imaging (MRI) scan and estimated values of the conductivity of various tissue types to give a conductivity value to each voxel of the scan; the position of the electrodes can then be added to the scalp and the path of the electrical current can be estimated (e.g. Neuling, Wagner, Wolters, Zaehle, & Herrmann, 2012). Although such models are only estimates, and individual differences (e.g. differences in

cortical folding) could result in significant differences in current flow (Laakso, Tanaka, Koyama, Santis, & Hirata, 2017), important insights can be gained (Berker, Bikson, & Bestmann, 2013). In particular, the idea that current flow is in one direction under the anode and the opposite direction under the cathode has been shown to be an oversimplification. The folded surface of the cortex means that current will flow into the wall of a gyrus and out of the other side, resulting in patterns of excitation and inhibition depending on the orientation of any one patch of cortex relative to the electrical field. Furthermore, the tangential and radial (relative to the skull) components of the electrical field will have different effects depending on the orientation of the underlying cortex, i.e. a sulcus or a gyrus (Caparelli-Daquer et al., 2012).

Electric field models can also be used to predict the effect of TES at the much smaller scale of individual neurons. Rahman and colleagues (2013) report how the flow of current in TES is primarily parallel to the cortical surface and the electric field can be described in terms of tangential and radial components relative to the somato-dendritic axis, which is tangential to the cortical surface in the case of layer 5 pyramidal cells (note: this is not the same as the tangential and radial components relative to the skull, as the cortex is folded). Because layer 5 pyramidal neurons have a more consistent orientation, i.e. long and thin, they are most susceptible to the gradient of an electrical field of the optimal orientation as the relative depolarization/hyperpolarization across the cell will be greater. The authors describe how the radial component will mainly have an effect via depolarization or hyperpolarization of the soma (the main body of the neuron from which the dendrites branch off), whereas the tangential component will function via depolarization/hyperpolarization of the synaptic terminals, although this relationship is more complex for other types of neuron in the cortex which can have more complex shapes (Rahman et al., 2013).

The relationship between the direction and intensity of electrical fields and the relative orientation and morphology of the affected neuron makes predicting the effect of TES and TMS at the level of individual neurons difficult. Behavioural effects as a result of electrical stimulation of the cortex are thought to be the sum of subtle effects on a large number of neurons, and therefore most likely to be the result of modulation of neurons with a similar orientation and morphology, e.g. layer 5 pyramidal neurons. However, there is the possibility that there exists a smaller subset of neurons, not in layer 5, which are more connected, or more easily affected by a change in electric field, and therefore are, to some extent, responsible for resulting behavioural effects.



## 2.4. Comparison of TMS and TES

Due to the amount of current delivered, the mechanism of action of TMS and TES are very different. TMS can deliver enough current to raise the membrane potential above the critical level to directly induce action potentials, whereas the weak currents used in TES only modulate the polarization of the membrane potential and thus the probability of neurons firing. However, this does not have to be the case:

TES can be used with high enough current to directly induce action potentials, but this current must first pass through the skin and as such is quite painful and is not suitable as a research tool. Likewise, TMS could be used with low enough intensities such as to interfere with on-going neural activity without directly inducing action potentials; this has not been explored to date but is an interesting possibility for future research. During my PhD I have conducted an experiment (in collaboration with the lab of Gregor Thut at the University of Glasgow) where we attempted to modulate alpha oscillations with constant 10 Hz TMS at very low intensities, similar to the current that reaches the cortex during TES, by positioning the coil 14 cm from the head; results did not show any significant effect, but future studies should explore this in more detail as it may be possible to combine the focality of TMS with the subtle effect of modulating, rather than driving, neural oscillations.

A significant advantage of TES over TMS is that the current flow is not limited to sudden bursts; TMS is generated via induction which is proportional to the rate of change of the magnetic field and as such requires a sudden change in field strength. Electrical stimulation can be delivered with any waveform shape or pattern; this has led to a number of variations on the classical direct current stimulation (although there are many unexplored possibilities, as I will discuss). Currently three types of TES, in addition to tDCS have been commonly used: transcranial random noise stimulation (tRNS), oscillating transcranial direct current stimulation (otDCS), and transcranial alternating current stimulation (tACS). tRNS involves a continuously changing current with random amplitude (Terney, Chaieb, Moliadze, Antal, & Paulus, 2008). The mechanism of action is not fully understood, but it has been hypothesized that the random stimulation enhances existing neuronal signals via stochastic resonance, a phenomenon by which a signal can be boosted by adding white noise as a result of the signal resonating with matching frequencies in the noise. The main focus of this thesis is tACS, which is a variant of TES in which the current switches from anode to cathode at a specific frequency. This technique will be discussed in detail below. Firstly I will discuss strategies for increasing the efficacy of all types of TES.

## 2.5. Optimizing Transcranial Electrical Stimulation

There are a large number of variations in the way in which TES can be delivered, all of which can be optimised to give the maximal effect. The focus of this thesis will be on exploring the possibilities of waveform shape in tACS, but other parameters which can be optimised include: electrode montage (i.e. the position of the electrodes on the head), electrode size/shape and type of connection to the scalp. Early studies using TES typically encased the silicon electrodes in sponges which were soaked in saline and held against the scalp with a strap to provide a good electrical connection. This is still commonly used today for many studies as it is quick to apply and does not require washing the hair after application. A second method is to use conductive paste, applied to the scalp and to the electrode, such that the two can be stuck together. This method has a number of advantages: firstly the conductive paste does not spread out beyond the application site, this is particularly important when combining TES with EEG, as using saline sponges can cause bridges to the EEG electrodes when the water soaks up into the cap or hair. Secondly, the fact that the conductive paste is confined to the application site makes this approach more precise. Thirdly, the conductive paste can provide more stable impedances; a problem with saline soaked sponges is that the water can dry out during the course of the stimulation causing higher impedances and requiring higher voltages to achieve the same current strength. This is an important issue for combining TES with EEG, which I will discuss further in the following chapters.

An aspect of TES methodology which has received attention in the literature is electrode size. Early studies typically used a standard 7 cm by 5 cm electrode, but more recently various experimenters have adopted variations on this standard. As the size of the electrode decreases the current density will increase for any given current. As the current density is considered to be critical to the effect on neural activity, smaller electrodes have been employed in an attempt to increase efficacy and focality. For example, many studies have used a smaller electrode above a site of interest and a larger electrode as a “return” electrode to minimise the current density in other cortical areas.

It should be noted here that more focal stimulation does not necessarily result in a greater effect on the cortex; some researchers have reported that 35 cm squared electrodes resulted in greater cortical excitation than 16 cm squared electrodes, and that the target (the motor hotspot for TMS) was predicted to have a higher electric field with the larger electrodes in computational models (Ho et al., 2016).

A significant amount of research has been done on increasing the focality of TES by using a number of smaller electrodes, rather than just two, often called High Definition-tDCS or HD-tDCS (Dmochowski, Datta, Bikson, Su, & Parra, 2011). A popular version is the 4 x 1 montage which involves one small stimulation electrode over the cortical target and four “return” electrodes surrounding it. Although these methods do increase the focality of TES significantly, as demonstrated with computational models and some experimental data (Caparelli-Daquer et al., 2012), there are a few limitations. Firstly, as the size of the electrodes decreases and the current intensity increases, the sensation on the scalp will become stronger, and at high intensities may become painful. This could be a problem for subject/patient discomfort and may limit the extent to which a sham condition (involving no stimulation) can be convincingly used. Secondly, an important factor to consider in all TES studies is the direction of the radial current, i.e. the current that flows between electrodes parallel to the skull, as opposed to the current that flows in or out perpendicular to the skull directly under the electrodes. This radial current is thought to contribute significantly to the effect of TES, and if this current is distributed in different direction towards multiple return electrodes the overall effect may not be comparable to conventional pairs of electrodes.

In the field of TMS research the problem of individual cortical variability has led to the development of neuro-navigated TMS in which a MRI scan of the participant’s brain can be converted to a 3D model, and combined with markers and an infra-red camera, to allow the exact position of the TMS coil to be seen and recorded. This can be combined with functional MRI (fMRI) to give the individual “hotspot” of maximal metabolic activation corresponding to the task for each individual, which can then be used as a target for stimulation. Neuro-navigation is not commonly used with TES, as it is not considered worthwhile given the relatively poor focality of TES. However, if accurate modelling of current flow combined with EEG source localisation during stimulation becomes technically feasible in the future, it may be fruitful to exactly localise the position of the stimulation and EEG electrodes relative to the individual structural MRI.

## **2.6. Targeting neural oscillations**

When faced with such a wide array of ways in which brain activity can be manipulated it is arguably best to focus on stimulation which mimics or compliments patterns of neural activity. Neural oscillations are increasingly being considered as fundamental to cognition, and as such, manipulating these oscillations is a promising research paradigm.

Rhythmic activity exhibited by neurons can range from 0.05 to 600 Hz (Buzsáki & Draguhn, 2004). Synchronised neural oscillations are thought to be the mechanism by which distant

brain areas communicate (Siegel, Donner, Engel, & Singer, 2012) and coherence of oscillatory neural signals is thought to be critical for multisensory perception (Senkowski et al., 2008, Keil & Senkowski, 2018). This is often referred to as the “Communication through Coherence” hypothesis. EEG oscillations consistently show larger amplitude in the lower frequency ranges and smaller amplitude as the frequency increases (the so called 1/f noise) and this is thought to reflect large populations of neurons mediating communication between distant cortical regions and increasingly smaller populations of neurons being recruited for local processing (Singer, 1993).

The most common methods for observing neural oscillations in humans are electroencephalography (EEG), in which recording electrodes are placed on the scalp to measure voltage differences resulting from neural activity, and magnetoencephalography (MEG) which uses magnetic sensors. The source of EEG/MEG is the simultaneous activity of large numbers of similarly orientated neurons; the source of EEG is thought to be predominantly the activity of pyramidal cells, found in layer 5 of the cortex, as these neurons are typically orientated in the same direction and the currents generated by post-synaptic potentials sum to create a voltage difference which is detectable at the scalp. For this reason, the signal recorded by EEG/MEG only reflects the activity of a subset of neurons in the cortex. The same principle most likely applies to currents applied to the cortex during TES; the voltage gradient applied to individual neurons is predicted to have the largest effect when polarizing the length of the neuron and the small effect on any one neuron will be multiplied if a large number of neurons are similarly orientated.

The ability to entrain or drive neural oscillations allows us to ask the question: are brain rhythms causally implicated in brain function or do they merely reflect by-products of other underlying mechanisms? Before tACS was available as a research tool a number of methods for modulating EEG oscillations existed, these include Steady State Evoked Potentials (SSEPs), EEG-neurofeedback and repetitive transcranial magnetic stimulation (rTMS).

SSVEPs are a subset of the more general class of steady state evoked potentials (SSEPs) which refers to the neural oscillations evoked by rhythmic input from any sense, for example an auditory train of clicks or a tone or even rhythmic tactile stimulation. SSVEPs can be seen in the EEG at frequencies ranging from 1-100 Hz, but show higher amplitudes in resonant frequency bands (alpha, beta, gamma etc.) due to an interaction with the preferred frequency of endogenous neural oscillations in the cortex (Herrmann, 2001).

The underlying mechanism of SSVEPs is not fully understood and there are two broad hypotheses, the entrainment of on-going neuronal oscillations and the superposition of event-related potentials (ERPs). The superposition of ERP model proposes that the

oscillations evoked by stimuli repeated at a particular frequency can be explained by the evoked activity from each single stimulus added together and interfering with the subsequent potentials (Capilla, Pazo-Alvarez, Darriba, Campo, & Gross, 2011). However, a more recent study looked at phase locking between on-going oscillations and visual flicker and found evidence of non-linear features that cannot be explained by a simple linear summation of single responses (Notbohm, Kurths, & Herrmann, 2016).

## **2.7. Transcranial alternating current stimulation**

The final type of TES which has been growing in popularity, and the focus of this thesis, is transcranial alternating current stimulation (tACS). Strictly speaking, tACS can include any TES in which the current changes polarity from anode to cathode at regular interval, but it is most commonly applied as a sinusoidal wave with no DC-offset (i.e. equal amounts of anodal and cathodal current under any one electrode), at frequencies matching the typical frequency range of endogenous neural oscillations. Oscillating transcranial direct current stimulation is essentially a variation of tACS, the only difference being that, due to the DC offset, the current does not alternate between cathodal and anodal. As the two methods are the same in all other regards, the following discussion of tACS also applies to otDCS.

TACS, and other oscillating/rhythmic stimulation such as otDCS and rTMS, have unique potential as neural oscillations are fundamental to how the brain functions and directly targeting neural-oscillations (as opposed to indirectly via sensory stimulation) allows for focused intervention at the level of the intrinsic mechanism, rather than simply disrupting or adding noise to the system.

The number of studies using tACS is already large and rapidly increasing; a PubMed search for articles containing the words "transcranial alternating current stimulation" or "tACS" in the title or abstract returned 222 published articles at the time of writing. A complete survey of the literature is beyond the scope of this introduction, but some key proof-of-principle papers will be summarized in the following paragraphs.

An important proof-of-principle finding was reported by Feurra and colleagues in 2011 (Feurra et al., 2011). These authors used motor evoked potentials (MEPs) from TMS, a commonly used measure of cortical excitability in which pulses of TMS to the primary motor cortex cause a muscle twitch in the hand which can be recorded using electromyography (EMG). The authors targeted 20 Hz (beta range) as oscillations in this frequency band are detectable in human sensorimotor regions during rest which attenuate or desynchronize during preparation and execution of movement. It was found that the MEPs (and therefore

the cortical excitability) significantly increased relative to baseline during the application of 20 Hz tACS. Importantly, the authors also tested a number of other frequencies (5, 10 and 40 Hz) with the same montage, duration and intensity and showed no significant effect on MEP size. This demonstrated that the effect of tACS on cortical excitability is specific to the frequency band of typical neural oscillations in the target area and most likely an interaction with the on-going oscillation. In addition, the authors conducted a control condition with 20 Hz tACS at another site (the parietal cortex) which also had no effect on MEP amplitude, confirming that the effect of tACS is somewhat confined to the targeted region of application.

Many studies that employ tACS target a specific frequency band which correlates with a behavioural measure of interest, based on existing EEG studies, and attempt to modulate the behavioural measure by stimulating at that frequency. Such studies can provide evidence of a frequency specific effect, but as they lack a measure of neural activity it is not possible to ascertain the mechanism by which tACS is having an effect, or to say for sure that the mechanism of action is entrainment of an on-going neural oscillation.

The first indication that tACS can directly modulate human EEG was reported in 2010 by Zaehle and colleagues who recorded EEG before and after tACS, which was delivered at participants' individual alpha frequency (Zaehle, Rach, & Herrmann, 2010). The authors found a significant increase in the amplitude of alpha oscillations after tACS compared to a sham group. This after-effect is thought to be due to entrainment of alpha oscillations leading to enhancement of networks of neurons which oscillate at approximately the same frequency via synaptic plasticity.

Direct evidence that external currents can entrain neuronal firing has come from a number of animal studies. In 2010, Ozen et al. demonstrated that neurons could be entrained to sinusoidal tACS in both anaesthetized and behaving rats (Ozen, Sirota, Belluscio, Anastassiou, Stark, & Koch, 2010); the authors reported neuronal spiking became phase-locked to the tACS and that the number of entrained neurons increased as the stimulus intensity increased. Additionally, the behavioural state of the animal also influenced the amount of entrainment. Around the same time Fröhlich and McCormick have reported intracranial stimulation of the cortex could entrain local field potentials (LFPs) and multiunit activity (MUA) (Fröhlich & McCormick, 2010).

The current dominant hypothesis for the mechanism of tACS is that the on-going neural oscillations are entrained to the frequency of stimulation. Although the entrainment and enhancement of on-going oscillations is a likely mechanism of action of tACS, other possibilities exist that should be considered. The findings of *in vivo* and *in vitro* experiments may not translate to the scale of human brain, or it could be the case that large populations

of neurons being entrained by distant brain areas are not susceptible to entrainment as the existing oscillation is too strong to be overcome (as implied by the findings of Lafon et al., 2017). For example, an alternative mechanism of action could be that the frequency and phase of the existing neural oscillations are unchanged but are enhanced by stimulation at nearby frequency as they “drift” in and out of phase with the external induced current. Another possibility is that the existing population of neurons that constitute the oscillation is unaffected by the stimulation and an additional population of neurons, not being driven by an external pacemaker and more susceptible to external driving force, forms a separate oscillation which can exist simultaneously, and interact with, the natural oscillation. Yet another possibility is that there is a property of neurons which is related to their tendency to synchronise at specific frequencies (e.g. the time constant of the flow of neurotransmitters or opening and closing of ion-channels), which might also be preferentially influenced by alternating electrical fields of the same frequency, which could affect neural activity in a frequency specific way although no entrainment actually takes place. To fully understand this mechanism more basic research needs to be done both in animals and in humans, an important part of which, I will argue, is the development of reliable simultaneous tACS and EEG.

An important model for explaining entrainment, borrowed from physics, is the concept of the “Arnold Tongue” which describes the relationship between an oscillator and an external driving oscillator: the greater the difference in frequency between the two oscillators, the stronger the force required for one to entrain the other. If neural oscillations follow the same pattern, the effect of tACS would be expected to increase as the frequency of the stimulation and neural oscillation get closer, and to be maximal when they are identical. An Arnold tongue relationship between tACS and EEG would provide evidence for the entrainment hypothesis.

## **2.8 Non-sinusoidal tACS**

TES is not limited to simple geometric shapes such as square, sawtooth or sine waves; it is also possible to embed multiple waveforms together to stimulate with “nested” oscillations. Furthermore, stimulation is not limited to repeating patterns; more elaborate stimulation can be delivered which, for example, follows the envelope of an audio sound. While such complex stimulation protocols may prove to be useful in future studies it is difficult to infer a relationship with on-going neural oscillations, which are by definition regular repeating patterns, and the combination of stimulation and neuroimaging is further confounded by such complex stimulation.

Evidence that non-sinusoidal waveforms, and in particular steeper gradients, may be better suited to modulate on-going neural oscillations comes from a study by Fröhlich and McCormick who investigated the required voltage needed to modulate neural activity in both *in vivo* and *in vitro*; they found different lower boundaries when stimulating with a sine wave compared to an electric field based on the on-going activity (Fröhlich & McCormick, 2010). In the supplementary material of the same study the authors applied electrical fields as ramps of increasing voltage and showed that steeper gradients resulted in increased neural firing, relative to ramps with a low gradient but which reached the same maximum voltage. This demonstrates that the rate of change of voltage is important for neural firing, and not only the total amount.

In the two studies presented in this thesis we chose to stimulate with sawtooth waves; in the first experiment with both positive and negative ramp sawtooth waves to compare the two, and as the results indicated a greater effect of positive ramp waves, this waveform was chosen for the second experiment.

## **2.9. Combining tACS with Neuroimaging**

Individual variability creates a need for personalised stimulation. TES is unlikely to reach its full potential until we can be sure that the stimulation parameters are optimised for the individual. Inter-subject variability is a significant problem for tACS (and all brain stimulation) but there is currently no established technique to individualise tACS intensity. The after-effect on alpha power is highly variable and only an indirect measure of entrainment. Simultaneous stimulation and recording of neural activity is necessary to fully understand the mechanism of action.

Individual variability exists in the time domain in the form of different frequencies; different individuals show variation in the dominant alpha rhythm between 8 and 14 Hz. In addition to the variability in individual alpha frequency (IAF), neural oscillations in other frequency bands also show variability across individuals, e.g. responses in the gamma range to auditory steady state potentials. Another significant source of variation is the individual cortical geometry; the unique pattern of sulci and gyri which, although show general similarities in overall form across individuals, can vary significantly in the exact shape and location of specific cortical features from one person to the next.

The ability to observe an online biomarker of the effect of tACS will be a valuable tool for the rapid prototyping of different stimulation montages, waveforms, intensities and electrode



types. This is important as there is a huge parameter space of unexplored possibilities as to how we can administer tACS, all of which can be individualised to give the maximum effect.

All of the primary methods of cognitive neuroscience for non-invasively imaging neural activity of human participants have been combined with tACS: EEG, MEG and fMRI. To date a few researchers have successfully combined tACS with fMRI (Vosskuhl, Huster, & Herrmann, 2016; Bächinger et al., 2017; Chai, Sheng, Bandettini, & Gao, 2018). This line of research is promising in some regards as fMRI has relatively good spatial resolution and allows the effect of tACS to be observed in deeper brain structures (unlike EEG/MEG which can only image the cortex). However, the temporal resolution of fMRI is typically in the order of tens of seconds, as it is the flow of oxygenated blood that is being recorded, not neural activity directly. To observe the effect of tACS on neural oscillations simultaneous EEG or MEG is needed.

A considerable problem for combined tACS-EEG is the large electrical artefact caused by the stimulation. As the majority of the current flows through the scalp (only a small percentage of the current reaches the cortex) the EEG electrodes are exposed to an electrical field which can be several orders of magnitude larger than the signal from neural activity. The artefact introduced in simultaneous tACS-MEG is smaller as there is no direct connection between the scalp and the magnetic sensors, but is still much larger than the desired neural signal due to the high sensitivity required of the sensors.

Various methods for removing the artefact have been used in recent studies, each with strengths and weaknesses (see discussion). In this thesis I will present an adaptive template subtraction method for removing the artefact, and in the discussion I will compare it to other methods.

The development of simultaneous TES and recording of EEG will be a useful research tool in its own right, but may also lead to the development of a more technically challenging goal of observing neural activity in *real time* by removing the artefact very quickly. This will allow for closed loop stimulation, which can react to on-going neural activity, e.g. an epileptic seizure, and stimulate accordingly.

## **2.10. Targeting Alpha oscillations**

The dominant oscillation in the human brain is the alpha rhythm, and as such is a good starting point for investigations into whether or not neural oscillations can be entrained by tACS. The alpha oscillation has received considerable attention. It is thought to function as a sensory suppression mechanism during selective attention (Foxe & Snyder, 2011) and to be involved in the ability to be consciously oriented in time and space (Klimesch, 2012). Alpha oscillations are approximately 10 Hz, although, as mentioned above, there is individual variation, typically between 8 and 14 Hz, and some individuals do not have an obvious alpha oscillation present in EEG recordings during rest with eyes-open.

Oscillations in the alpha band are the focus of the two studies presented in this thesis but in two different ways; in the first study spontaneous, resting state, alpha band oscillations are the focus, in the second experiment SSVEPs in the alpha band are observed. There are considerable differences between naturally occurring oscillations and SSVEPs and there is an on-going debate in the literature as to the relationship between the two (see discussion). The goal of this research is to use tACS to manipulate neural oscillations in a reliable way, regardless of whether they are the naturally occurring or driven by external rhythmic stimuli. However, the methods presented in this thesis may prove useful in addressing this question by testing if endogenous neural oscillations react in similar, or dissimilar, way to SSEPs when modulated with tACS (see discussion).

## 2.11. Overall goals of this thesis

The research questions of the work presented here can be summarized as:

- Is tACS modulating neural oscillations?
- Can we get a reliable biomarker of tACS to help optimise and guide the development of the method?
- Specifically, can we remove the artefact and see EEG during stimulation?
- Is there a difference in the effect of tACS with different waveforms when all other factors (frequency, amplitude) are controlled for, specifically comparing positive and negative ramp sawtooth waves?
- How close in frequency do neural oscillations need to be to the tACS frequency for there to be an effect?
- How high a current intensity is needed to manipulate neural oscillations during ideal frequency matched stimulation?

In addition to the main question of optimizing the method of tACS, the second study also had the goal of investigating SSVEPs as a potential biomarker of vection (the feeling you are moving when you are not) and evidence for lateralization of cortical function during visual-vestibular integration. When exposed to large, full-field optic flow, information from the visual and vestibular systems (as well as somatosensory and proprioceptive systems) must be combined in order to determine whether object- or self-motion is occurring. The second study in this thesis induces vection as a means to explore frequency specific intervention. Given that alpha oscillations have been shown to be important for multisensory perception, this frequency band was targeted with tACS. Further, given evidence to suggest that visual-vestibular integration is lateralized, with bilateral vestibular information being combined in the right hemisphere of right-handers, this study also examines the role of lateralization of visual-vestibular integration.

# Transcranial alternating current stimulation with sawtooth waves: simultaneous stimulation and EEG recording

**This chapter was published\* as:**

Dowsett J and Herrmann CS (2016) Transcranial Alternating Current Stimulation with Sawtooth Waves: Simultaneous Stimulation and EEG Recording. *Front. Hum. Neurosci.* 10:135. doi: 10.3389/fnhum.2016.00135

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## 3.1. Abstract

Transcranial alternating current stimulation (tACS) has until now mostly been administered as an alternating sinusoidal wave. Despite modern tACS stimulators being able to deliver alternating current with any arbitrary shape there has been no systematic exploration into the relative benefits of different waveforms. As tACS is a relatively new technique there is a huge parameter space of unexplored possibilities which may prove superior or complimentary to the traditional sinusoidal waveform.

Here we begin to address this with an investigation into the effects of sawtooth wave tACS on individual alpha power. Evidence from animal models suggests that the gradient and direction of an electric current should be important factors for the subsequent neural firing rate; we compared positive and negative ramp sawtooth waves to test this.

An additional advantage of sawtooth waves is that the resulting artefact in the electroencephalogram (EEG) recording is significantly simpler to remove than a sine wave; accordingly we were able to observe alpha oscillations both during and after stimulation.

We found that positive ramp sawtooth, but not negative ramp sawtooth, significantly enhanced alpha power during stimulation relative to sham ( $p < 0.01$ ). In addition we tested for an after-effect of both sawtooth and sinusoidal stimulation on alpha power but in this case did not find any significant effect.

This preliminary study paves the way for further investigations into the effect of the gradient and direction of the current in tACS which could significantly improve the usefulness of this technique.

### 3.2. Introduction

Transcranial alternating current stimulation (tACS) is increasingly being used as both an investigational tool and for clinical intervention as it can modulate cortical activity in a frequency specific manner and is thought to function by entraining neural oscillations. A number of studies have shown that tACS at alpha frequencies can enhance alpha oscillations (Helfrich et al., 2014; Neuling, Rach, & Herrmann, 2013; Zaehle et al., 2010). The current study continues this line of research by observing the effect of 10Hz tACS on alpha power.

A provisional explanation for the frequency specific effects of tACS is that on-going neural oscillations are entrained to the electrical stimulation. One mechanism by which this might happen is that the applied electrical field modulates the local field potential such that the positive (anodal) phase of the stimulation increases the likelihood of neuronal spiking and the negative (cathodal) phase decreases the likelihood. As a result the on-going neural oscillations may become synchronized with the alternating current; this has been shown to be the case in both recordings from cortical slices stimulated with an electrical field (Fröhlich & McCormick, 2010), and in intracranial recordings in rats stimulated with electrodes on the surface of the skull (Ozen, Sirota, Belluscio, Anastassiou, Stark, Koch, et al., 2010).

The majority of tACS studies to date have used a sinusoidal waveform, however an alternating current does not have to be sinusoidal; it can be a square wave, triangular, pulsed or any arbitrary waveform. There have been a few exceptions to the convention of using sinusoidal waves for alternating or oscillating transcranial stimulation which have shown interesting results, for example pulsed current stimulation has been shown to affect corticospinal excitability (Jaberzadeh, Bastani, & Zoghi, 2014) and slow wave rectangular stimulation has been shown to have an effect on memory consolidation during sleep (Marshall, Helgadóttir, Mölle, & Born, 2006).

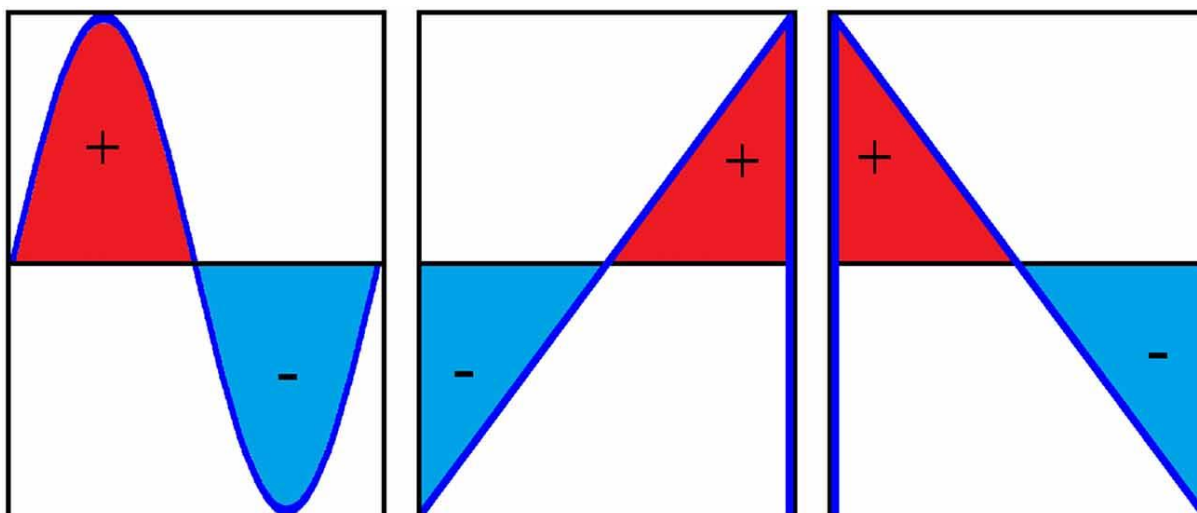
There are various reasons why steep or instantaneous changes in current, such as in square waves or sawtooth waves, might be better suited to entraining on-going neural oscillations. Fröhlich and McCormick (2010, supplemental material) have shown that ramps of increasing voltage with a steeper gradient resulted in increased neural firing *in vitro*, relative to ramps with a low gradient but which reached the same maximum voltage. This demonstrates that it is not only the total amount of current but also the rate of change of current which modulates neural firing.

To understand how electrical fields might entrain neural oscillations it is important to consider the mechanism behind different cortical rhythms. Reato et.al (2013) discuss how slow wave neural oscillations consist of a period of high activity followed by an inactive period; the

duration of the high activity state is thought to be determined by the depletion of cellular resources and cannot be easily changed, whereas the duration of the low activity state can be more readily modulated and under certain conditions can be ended by a single spike at the optimal time resulting in a cascade of firing which begins at the next cycle of the oscillation. A relatively weak external electric current, with the optimal polarity and at the critical point in time, would be sufficient to initiate the onset of the active state, and when repeated at the right frequency might drive or entrain on-going activity. We can speculate that a sudden change in current would be more suited to this role than the relatively slow rise of a sine wave if the transition from one state to another depends on a sufficient number of neurons firing together at a critical time.

By administering transcranial electrical stimulation with waveforms such as square wave or sawtooth waves the maximum rate of change of current flow at the cortex becomes more similar to other brain stimulation techniques such as transcranial magnetic stimulation (TMS) where the current flow in the cortex steeply rises and falls in less than a millisecond, although the mechanism of action is completely different; TMS is super-threshold, directly inducing action potentials whereas tACS is subthreshold, influencing the probability of action potentials. In addition, electroconvulsive therapy (ECT) is known to be significantly more efficient at inducing seizures with lower electrical charge using square waves rather than sine waves (Abrams, 2002); again the mechanism of action is entirely different, but if a sudden change in current is more effective than a sinusoidal current at causing neurons to fire it is not unreasonable to assume that the same is true for the subthreshold effect of much weaker currents on the probability of neurons firing.

In the current study we chose to compare tACS with positive ramp and negative ramp sawtooth waves (figure 1, example EEG data in figure 2). A sawtooth wave consists of two distinct components: the linear ramp during which the current gradually changes over 100ms (with 10 Hz stimulation), and the vertical transition where the current switches direction instantaneously. We chose to use sawtooth waves to differentiate between the effect of a sudden jump in polarity at the Oz electrode from anode to cathode in the case of positive ramp, and from cathode to anode in the case of negative ramp. A square wave would contain sudden transitions in both directions.



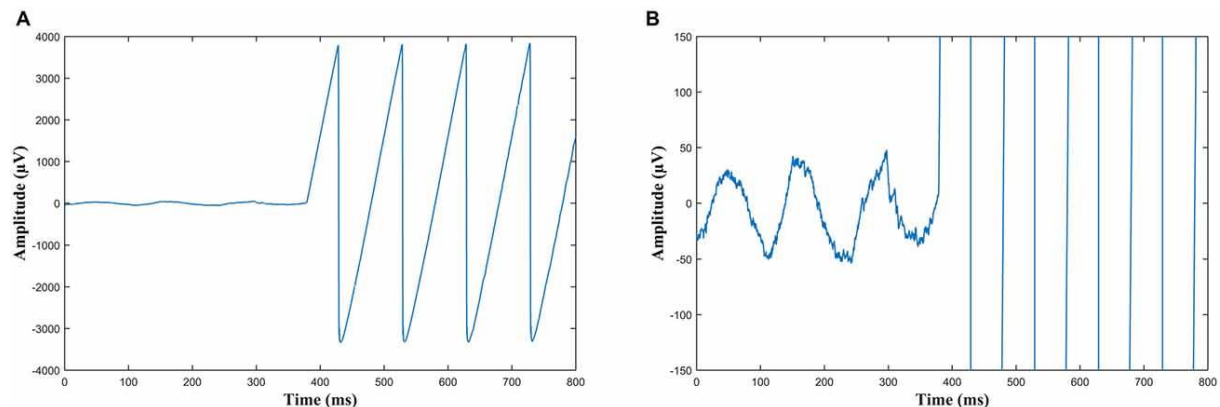
*Figure 1: One cycle of a sine wave, positive ramp sawtooth and negative ramp sawtooth (from left to right). Positive and negative ramp sawtooth waves contain identical amounts of positive and negative charge, i.e., the area under the curve is the same.*

It is known from TMS studies that changing the current direction (by rotating the orientation of the coil) can have significantly different effect on the neural response. This variation has been shown in the motor cortex to be generally consistent across the majority individuals, while a minority show a different optimal direction (Balslev, Braet, McAllister, & Miall, 2007). This is thought to be due to different populations of neurons being activated preferentially by different current directions. Variation in current direction has also been shown to affect TMS phosphene threshold; lateral to medial induced current in the visual cortex is optimal to induce phosphenes (Kammer, Beck, Erb, & Grodd, 2001). Interestingly, a recent study has shown that TMS evoked alpha oscillations, generated with the TMS coil held vertically such that the significant induced current in the cortex flows in the anterior–posterior direction, show the same pattern of variation in amplitude due to attentional shifts as spontaneous alpha oscillations (Herring, Thut, Jensen, & Bergmann, 2015); this current direction is comparable to the tACS in the current study, i.e. flowing between Oz and Cz.

If current direction and gradient are important, we hypothesized that positive and negative ramp sawtooth would have a different effect on alpha power. Conversely, if there were no difference in cortical activity this would suggest that the gradient of the current is irrelevant and any effect is simply due to the alternating periods of positive and negative current.

A further advantage of sawtooth waves is that the resulting artefact in the EEG recordings during stimulation is simpler to remove; the distinct properties of sawtooth waves, i.e. consisting of straight lines with a steep transition, do not occur in nature and as such are easily distinguishable from neural activity, especially in the frequency domain where they show characteristic harmonics. As such it is possible to be sure that no residual artefact

remains in the cleaned data. It should be noted that this is also true for square waves which could also be analysed in this way in future studies.



*Figure 2: (A) An example of the onset of sawtooth wave Transcranial alternating current stimulation (tACS) recorded in EEG from electrode Pz. Note the sawtooth waves are slightly rounded at the peaks due to capacitance. (B) The same data as (A) but with the scale adjusted such that the on-going alpha oscillations can be seen before the stimulation starts and are obscured during stimulation, which at this scale appears as near vertical lines.*

### 3.3. Methods

#### *Experimental procedure*

Thirty healthy subjects (16 female) with a mean age of 25 (max: 30 min: 19) participated in the study. Participants gave written consent after being fully informed as to the experimental procedure. All participants self-reported as being right handed and free from neurological or psychiatric diseases. The experimental protocol was approved by the local ethics committee.

Each participant came into the lab on four separate days and received a different condition on each day: sinusoidal tACS, positive ramp sawtooth tACS, negative ramp sawtooth tACS and sham stimulation. The order of conditions was randomized. EEG was recorded for 5 minutes before stimulation, during the 10 minutes stimulation and for 5 minutes post stimulation.

All tACS had peak-to-peak amplitude of 2mA and was administered from a stimulator with the option of delivering current controlled by a remote input (Eldith, Neuroconn, Germany); the waveforms were generated in MATLAB (The MathWorks Inc., Natick, MA, USA) at 5000Hz and sent to the stimulator via a digital-to-analog converter (National Instruments USB-6229 BNC). The stimulating electrodes were a 4 x 4 cm electrode centred on Oz and a 5 x 7 cm electrode centred on Cz. These sizes were chosen to give a higher current intensity



over the occipital cortex, as this is thought to be a source of alpha oscillations, and a lower current intensity over Cz which is not thought to be involved in the generation of alpha oscillations. The polarity of the stimulation was such that when the input waveform was positive the electrode at Oz was anodal and Cz was cathodal, and vice versa in the negative half of the wave. All tACS was delivered at 10Hz.

In the sham condition stimulation was delivered at full power (sine wave) for 10 seconds and then faded to zero over a further 10 seconds. Pilot data was collected from three lab members who reported that they could feel the sensation of tACS at onset but could no longer feel the on-going stimulation after one minute, and could not distinguish between this and the sham condition, this suggested that this procedure is sufficient to induce the sensation of stimulation which persists for longer i.e. participants cannot tell when the stimulation ends.

On each experimental session the tACS electrodes were attached using a conductive paste and the impedance was measured to insure it was below 10k ohms (in most cases it was below 5k ohms). Next, the EEG cap was fitted over the tACS electrodes and five recording electrodes were set to the parietal sites (P7, P3, PZ, P4 and P8) according to the 10–20 System. The EEG was amplified using a BrainAmp amplifier (Brain Products, Munich, Germany). Impedance of the EEG electrodes was kept below 10k ohms and was recorded with a sampling rate of 5000 Hz (the same as the tACS signal). The reference electrode was attached to the tip of the nose and a further electrode was placed below the right eye to record eye movements. The ground electrode was positioned on the forehead at electrode position Fpz. The experiment was performed in an electrically shielded, sound-proof, and dimly lit room (Vacuumschmelze, Hanau, Germany).

The experiment was double blinded in as much as the experimenter, who attached the tACS electrodes, fitted the EEG cap and explained the procedure to the participant was not aware of the stimulation the participant would receive; the order of conditions was determined at random by the computer controlling the experiment and only observed by a second experimenter.

Throughout the entire experiment (pre, stimulation/sham and post EEG) the participants were instructed to fixate on an LED and press a response button whenever it illuminated to insure a consistent level of vigilance. The LED illuminated at random intervals between 50 and 60 seconds. We chose to record with eyes open and not with eyes closed because a previous study (Neuling et al., 2013) has shown an increase in alpha power after tACS with eyes open but not with eyes closed, so it would seem that tACS does not have a significant effect on eyes-closed alpha power, perhaps because of a ceiling effect.

After each experimental session participants were given a questionnaire to assess any possible adverse effects (Neuling et al., 2013) which asked about any of the following symptoms: headache, neck pain, scalp pain, tingling, itching, burning sensation, skin redness, sleepiness, trouble concentrating and acute mood change. Participants were asked to indicate the intensity of the side effect (1, absent; 2, mild; 3, moderate; 4, severe) and if they attributed this to the tACS. Additionally they were asked on each day if they felt the stimulation and if so for how long they thought the stimulation lasted. Participants were also asked if they perceived phosphenes. The results of these questionnaires were collected and analysed.

As an additional analysis, to test the artefact removal method, a 10Hz sawtooth wave of comparable size to a typical artefact was generated in MATLAB and added to the ten minutes EEG recording from the sham condition (excluding the 20 seconds stimulation) for each participant, the artefact was then removed using the same procedure (described below) and compared to the raw data.

### *Data analysis*

Electrode Pz was initially selected for amplitude analysis as in previous studies (Neuling et al., 2013). For some of the participants Pz could not be used for the online analysis as the tACS artefact was too large and caused the signal to clip, rendering the data unusable, as such electrode P4 was used, as this was the only electrode not corrupted in all participants and all conditions. The same electrode was used across all conditions. While there is the possibility that any effect found might only be in the right hemisphere it is unlikely as the stimulating electrodes were positioned on the mid-line and we would expect the current to reach both hemispheres equally.

Of the thirty participants tested, twelve had no observable peak in the alpha band above 1/f noise in either the pre or post measurement. If there is no observable alpha activity we would not be able to see any change in alpha power and as such these participants were not included in any further analysis. Experience from other studies has shown that it is not uncommon for such a high percentage of participants to have no observable eyes-open alpha peak, for example Min et al. (2007) found that 8 out of 23 subjects had no detectable alpha peak, this is a similar ratio as found in the current study (12 out of 30). Other studies (e.g. Smit et al. 2006) have found a significant number of individuals showing no alpha peak, although a lower percentage than found in the current study. It is unfortunate that such a high number had no detectable alpha peak, however this data is still useful as we were able to

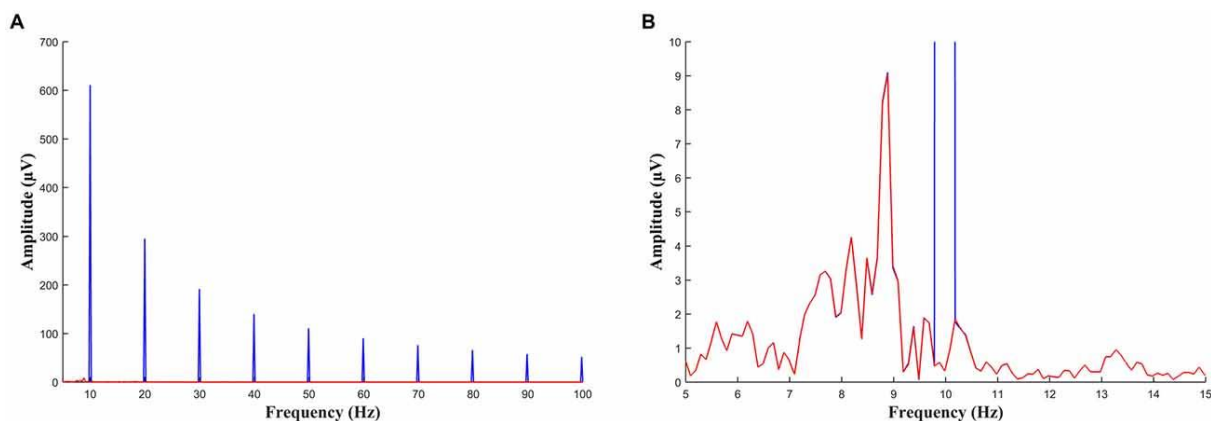
remove the artefact and show that there is no residual peak at 10Hz, demonstrating that the artefact removal method does not leave a residual artefact (see discussion).

Of the eighteen remaining participants three were rejected from further analysis due to excessive noise caused by the tACS or excessive muscular artefacts making further analysis impossible; as a result fifteen participants (six female) were included in all subsequent analyses.

Artefact removal and pre-processing was performed with MATLAB and statistical tests were performed with R (R Foundation for Statistical Computing, Vienna, Austria).

### *Artefact Removal*

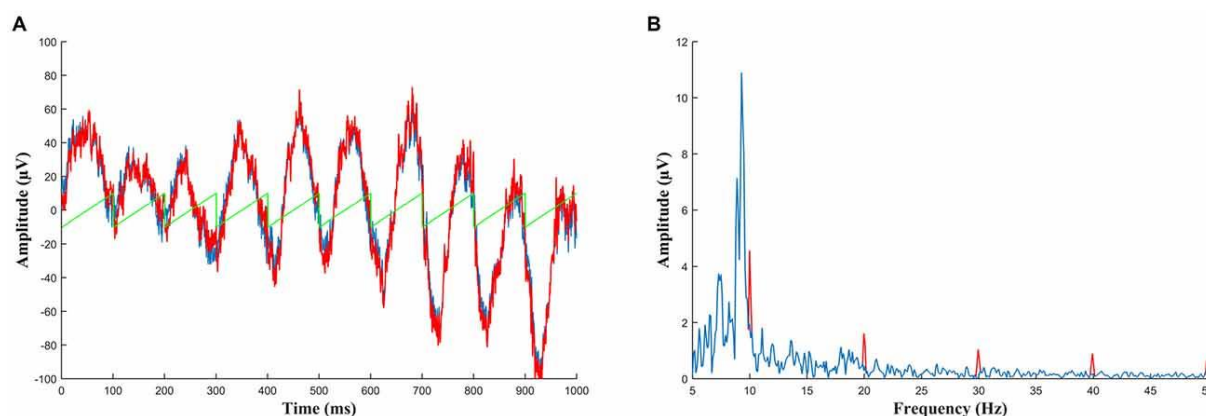
The tACS artefact was removed using a modified version of a template subtraction technique used by Helfrich et al.(2014), a critical difference in the current study is that a template of ten seconds was used to give a higher frequency resolution. The EEG recording during stimulation was first divided into ten second segments. For each segment a ten second sliding window was used, starting from ten seconds before the period to be analysed and moving forward in 100ms steps (the length of one 10Hz tACS oscillation) for 200 steps; these windows were then averaged to create a template of the artefact and subtracted from the original ten second window (see figure 3 for example data).



*Figure 3: (A) FFT of a 10 s segment of EEG during sawtooth tACS before (blue) and after (red) the artefact has been removed. Large peaks at 10 Hz and at all harmonics of 10 Hz can be seen before the data is cleaned. (B) The same data as (A) with the scale adjusted such that the alpha peak can be seen. This participant had an individual alpha frequency of approximately 9 Hz. The data before and after artefact removal are virtually identical except for the peak at 10 Hz which has been removed in the cleaned signal (red).*

The tACS stimulator delivers a constant peak current and as a result any changes in impedance will result in changes in the size of the artefact in the EEG recording (measured in micro-volts). Generally there is a gradual change in the size of the artefact over time due to the electrode gel drying out, participant sweating etc. In addition there are occasionally sudden jumps in the size of the artefact, most likely due to participant movement. For this method to work it is important that the tACS artefact does not change size suddenly during the period used to create the sliding window as this will result in an incorrectly sized template and a residual artefact in the cleaned data.

A number of steps were taken to insure against this by rejecting any segments for which the artefact was not correctly removed. Firstly, the template was created by averaging only sliding windows for which the amplitude at each data point was less than 200 $\mu$ V above or below the amplitude of the segment to be cleaned. Secondly, before the template was subtracted, an FFT was performed on the template itself and it was not used if it contained activity at any frequency other than 10Hz and harmonics (20Hz, 30Hz ... etc.); this ensured that only consistent activity at exactly the stimulation frequency ( $\pm$  0.05Hz) would be subtracted (as a ten second segment was used the resulting FFT had a resolution of 0.1 Hz). As a third step, the cleaned ten second segment was rejected from any further analysis if it contained any evidence of residual artefact. A distinctive characteristic of sawtooth waves is that they contain strong harmonics when viewed in the frequency domain; with 10 Hz stimulation a sawtooth wave would show strong peaks at every multiple of 10Hz (figure 3a). Any ten second segment which contained peaks at any multiple of 10Hz above 20Hz, greater than one standard deviation above the average level of noise in the adjoining  $\pm$ 5Hz range, was rejected from further analysis (demonstrated with simulated data in figures 4a and 4b). Although this is probably an overly conservative criterion (i.e. occasionally segments with no residual artefact but high levels of noise would have been rejected) it was selected to be certain that no residual artefact remained. The 20Hz harmonic was not included in the rejection criteria as some participants showed beta peaks around 20Hz in the pre-measurement and as such 20Hz peaks could conceivably be entrained beta activity. Using a ten second segment is preferable for this step as the harmonics which result from residual sawtooth artefact are more clearly visible above noise.



*Figure 4: (A) Simulated data to demonstrate detection of a residual artefact. Ten seconds of baseline EEG (in blue, only 1 s shown) was added to a small sawtooth wave with amplitude of 10  $\mu$ V (green) to create a corrupted signal (red) such as is seen when an incorrectly sized template is subtracted during artefact removal. Viewed in the time domain this signal cannot be differentiated from normal EEG. (B) The same data as (A) but viewed in the frequency domain. Here the corrupted signal (red) can easily be identified by harmonics which stand out above the level of noise. The peak at 10 Hz could potentially be entrained alpha oscillations but the other harmonics above 30 Hz (which continue throughout the frequency plot) indicate the presence of a sawtooth artefact. Therefore any segment which shows this activity should be rejected from analysis.*

It should be noted that this method would not work with the sinusoidal stimulation as any residual artefact would only contain activity at 10 Hz with no harmonics and as such is not distinguishable from EEG at 10Hz using only one electrode. For this reason, and because there were insufficient EEG electrodes for other artefact removal techniques such as PCA, the online data for the sinusoidal tACS was not analysed as there would be no criteria for determining if the artefact had been fully removed.

As a final step the cleaned ten second segments were further divided into one second segments and any containing eye blinks or muscular artefacts were rejected.

### *EEG analysis*

The analysis of the cleaned online data, the offline data (the pre and post measurements) and the cleaned “simulated artefact” data was carried out using a modification of a method used by Zaehle et al. (2010). EEG data was split into one second segments, if a segment included an eye blink or muscular artefact it was rejected from further analysis.

The first 200 artefact free one second segments for pre, online and post for each condition were baseline corrected by subtracting the mean, multiplied by a hanning window, and an FFT was applied to each. The resulting FFT spectra were then averaged.

For each averaged spectra the peak value was taken as the maximum between 8 and 14Hz. The amplitude of the alpha was taken as the mean of the range  $\pm 2$ Hz from this peak. To account for individual/inter-trial variation in alpha amplitude each online and post alpha amplitude value was normalized relative to the average amplitude from the corresponding five minute pre measurement. These relative values were then subjected to statistical analysis.

For the online data a repeated measures ANOVA with one factor of condition and three levels (positive ramp sawtooth tACS, negative ramp sawtooth tACS and sham) was performed on the normalized alpha amplitude values. For the post data a repeated measures ANOVA with one factor of condition and four levels (sinusoidal tACS, positive ramp sawtooth tACS, negative ramp sawtooth tACS and sham) was performed on the normalized alpha amplitude values. Post-hoc pairwise t-tests with Bonferroni correction were carried out to compare conditions.

In addition, a repeated measures ANOVA with four levels was applied to the mean peak alpha amplitude of the five minutes pre-measurement for each condition to test for any significant differences between conditions (as each condition was recorded on separate days and alpha power can change from one day to the next).

### 3.4. Results

#### EEG data

EEG spectra comparing the amplitude of the alpha frequency band prior to stimulation to online data during stimulation (before normalization) are shown in Fig. 5. For the online data a repeated measures ANOVA of the normalized alpha amplitudes revealed a significant effect of condition ( $F_{2,28} = 8.4735$ ,  $p = 0.0013$ ). Pairwise t-tests (Bonferroni corrected) showed a significant difference between positive ramp sawtooth and sham ( $p = 0.0059$ , cf. Fig 7), but no significant differences between any other conditions ( $p > 0.1$ ).

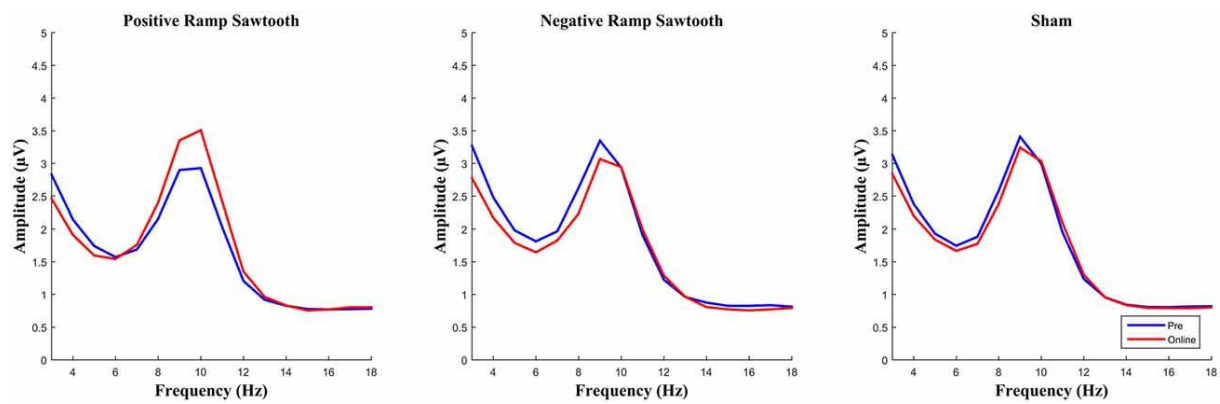


Figure 5: Average spectra for all participants for pre measurement (blue) and online (red), before normalization. Only the positive ramp sawtooth yielded a significant difference from sham after normalization.

EEG spectra before normalization comparing the amplitude of the alpha frequency band prior to stimulation to post stimulation are shown in Fig. 6. For the post data a repeated measures ANOVA of the normalized alpha amplitudes showed no significant effect of condition ( $F_{3,42} = 2.01$ ,  $p = 0.126$ ). Pairwise t-tests (Bonferroni corrected) showed the difference between positive ramp sawtooth and sham to be approaching significance ( $p = 0.098$ ), whereas  $p > 0.5$  for all other condition pairs.

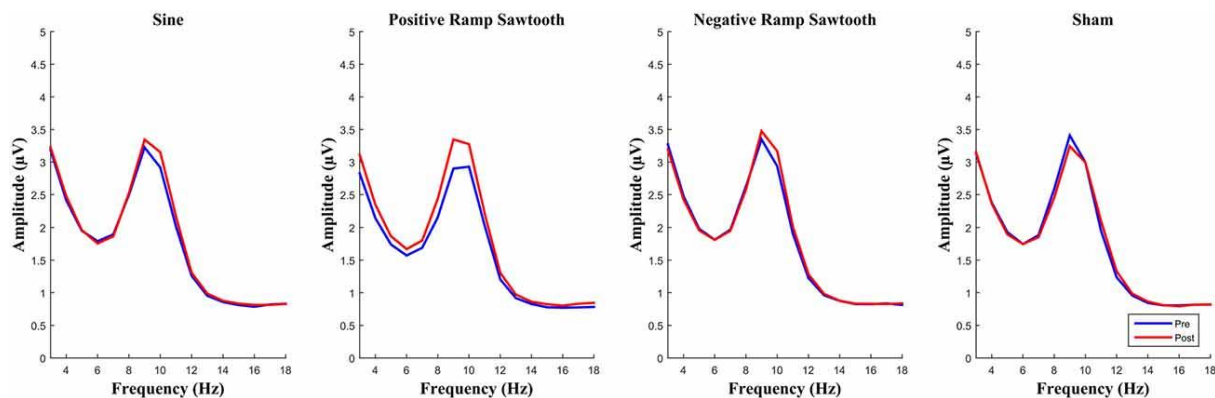


Figure 6: Average spectra for all participants for pre (blue) and post (red) measurement, before normalization. None of the differences between conditions reached significance after normalization.

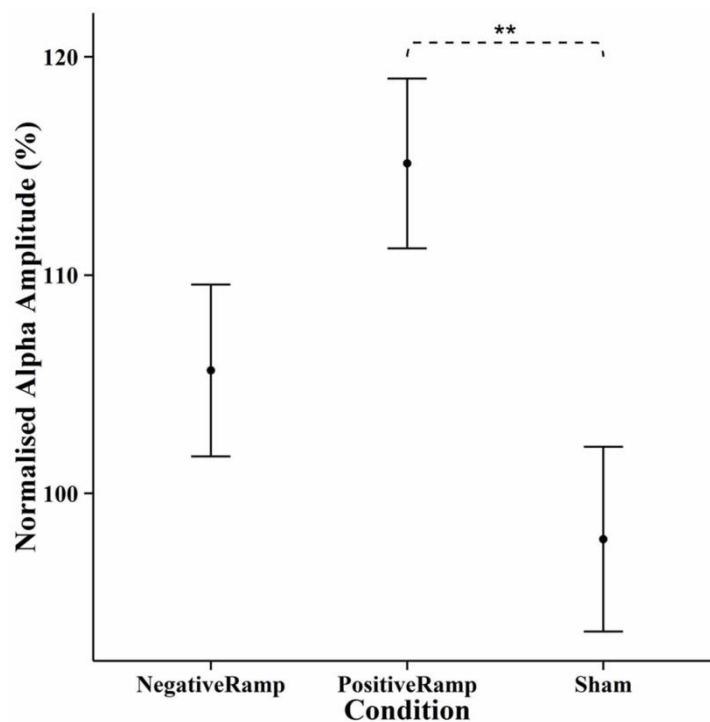


Figure 7: Normalized mean alpha amplitude online for each condition, error bars show  $\pm 1$  standard error of the mean. Stimulation with positive ramp yielded a significantly stronger amplitude of alpha oscillations during stimulation compared to sham. “\*\*” indicates  $p < 0.01$ .



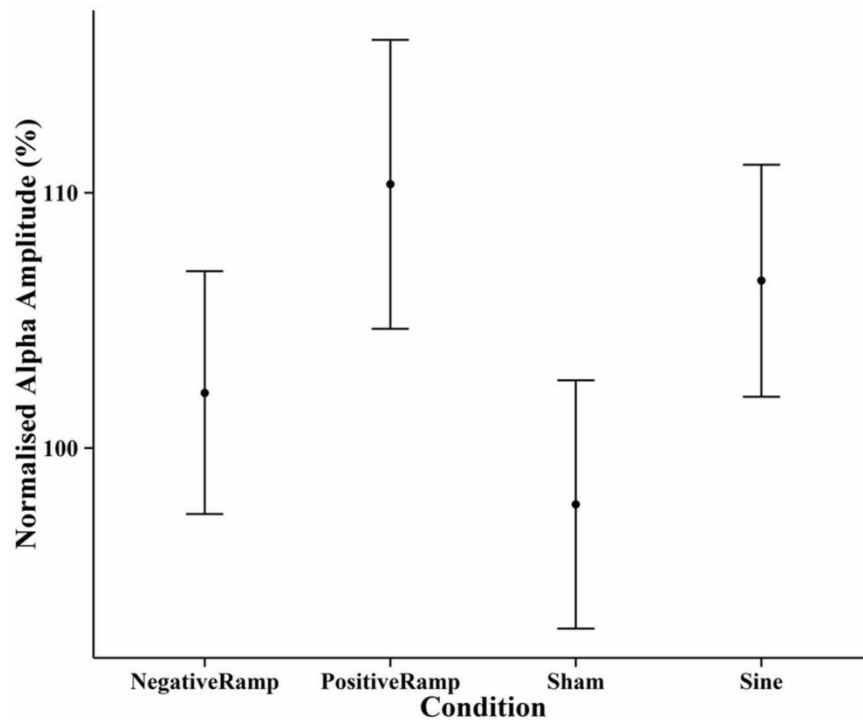


Figure 8: Normalized mean alpha amplitude post-stimulation for each condition, error bars show  $\pm 1$  standard error of the mean. None of the differences between conditions reached significance for the pre-post comparison.

The repeated measures ANOVA comparing the mean alpha peak of the four pre-measurements showed no significant difference between the four conditions ( $F_{3,42} = 0.045$ ,  $p = 0.987$ ).

For the simulated artefact test the raw EEG from the sham condition was compared to the same data with a sawtooth artefact added and then removed; the resulting mean spectra were identical at all frequencies except 10Hz where there were slight differences ( $<1\%$ ). The pairwise linear correlation coefficient between every cleaned one second segment of EEG data and the corresponding original data was calculated, the mean correlation was 0.97. The alpha peak of the mean FFT of the cleaned data was always either identical or slightly lower than the alpha peak of the original EEG, the mean error was  $0.015\mu\text{V}/\text{Hz}$  lower (the maximum error was  $0.07\mu\text{V}/\text{Hz}$  lower). Importantly, any error was always below the true value (because activity at 10Hz is removed) and as such the increase in alpha amplitude found in the real data would at worst be an underestimate i.e. the true alpha power might be slightly higher.

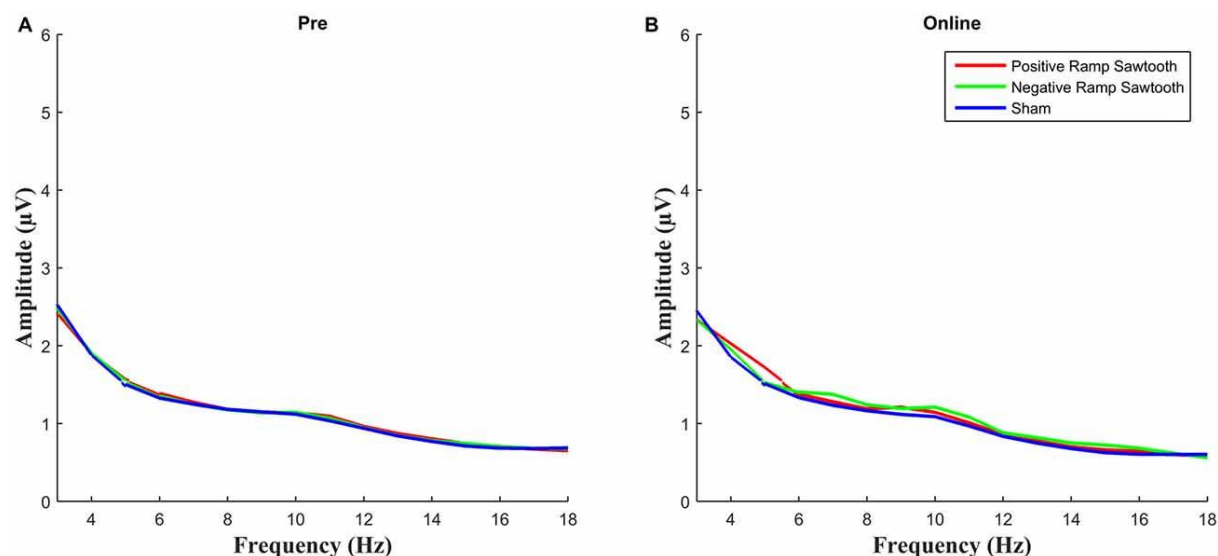
### Questionnaire

All thirty participants were used for the analysis of the side-effects and sensation reports. Individual responses to each item on the questionnaire for each condition were entered into a Friedman test; there was no significant effect of condition for any of the side-effects ( $p > 0.1$  for all). The most common reported sensations were Itching, Tingling and Heating (mean scores for all conditions  $< 2$ , i.e. mild sensation). When asked to estimate how long the stimulation lasted 17 of the participants reported that they felt the stimulation for under a minute in all conditions, 5 reported the sensation of stimulation throughout the experiment in all conditions, 4 reported no sensation at all in any of the conditions and 3 were able to distinguish between the sham and stimulation conditions reporting sensation throughout the experiment in all conditions except sham. Therefore, all but three participants were successfully shamed in one way or another. Only these three participants reported seeing phosphenes throughout the experiment, there was no difference between the reports of phosphenes between any of the stimulation conditions. Importantly the side effect scores and estimates of stimulation duration were almost identical for positive and negative ramp sawtooth stimulation for all participants; as such the main finding of a difference between positive and negative ramp sawtooth waves (compared to sham) cannot be attributed to skin sensation or phosphenes (see discussion).

### 3.5. Discussion

The primary aim of this study was to compare the effect of positive and negative ramp sawtooth wave tACS on alpha oscillations. The fact that it is possible to tell whether artefacts from non-sinusoidal tACS have been successfully removed is an additional advantage. Our main finding was that positive ramp sawtooth stimulation significantly increased alpha power during stimulation relative to a baseline condition, whereas negative ramp sawtooth did not. The positive and negative ramp sawtooth waves were identical in terms of frequency, peak current and total charge delivered (i.e. the derivative of current by time in coulombs); this indicates that the gradient of the current and the current direction play an important role in the modulation of on-going alpha oscillations. As a sudden change in current is more likely to have an effect than a gradual change we can hypothesize that the steep change from 1mA anodal to 1mA cathodal at electrode Oz every 100ms is likely to be the primary cause of the increase in alpha power found here. As both positive and negative ramp sawtooth waves contain a sudden change in current direction we can conclude that it is a sudden change in current in the optimal direction which is causing the effect.

Participants with no observable peak in the alpha range did not show any peak during stimulation after the artefact was removed (figure 9); this is further evidence that the artefact removal method does not leave any residual artefact.



*Figure 9: Average spectra for all participants who showed no alpha peak in all conditions: (A) for the 5 min pre measurement, (B) for the 10 min of stimulation after the artefact has been removed.*

Unfortunately we were not able to directly compare the online effect of sinusoidal tACS with the two sawtooth tACS conditions. Recent studies have had some success removing the artefact resulting from sinusoidal tACS in EEG (Helfrich et al., 2014), using a combination of template subtraction and PCA to remove any residual artefact, and in MEG (Neuling et al., 2015) using beamforming. However, it should be noted that careful observation of the size of the artefact in the EEG from the current study reveals that as well as the artefact changing size over time it can in some cases change in a different direction across electrodes (i.e. shrink in one electrode and grow in another) thus changing the topography of the artefact at the scalp; this would not be immediately obvious and as such caution should be used when interpreting data where a sinusoidal tACS artefact has been removed from EEG, even after PCA has been used to remove any residual artefact.

The ability to remove the tACS artefact from a single electrode (albeit by rejecting corrupted segments) is an advantage as it is simpler setup and may be more desirable in some situations, for example in clinical settings where a full cap of 64 EEG electrodes is not practical.

In the test of the artefact removal method the simulated sawtooth artefact was removed almost perfectly from the 10 minutes EEG recording with only a slight loss at the stimulation frequency. This illustrates a drawback of the template subtraction method as used here:

neural oscillations at exactly the stimulation frequency can also be included in the template and subtracted. As we used a 10 second template, only constant oscillations between 9.95 Hz and 10.05 Hz would be affected. There could potentially be neural oscillations entrained to exactly the stimulation frequency that would be lost. This can be demonstrated by adding a simulated artefact at 10Hz to EEG data containing a steady state visually evoked potential (SSVEP), also at exactly 10 Hz, and removing the artefact; in this case the SSVEP would be lost (data not shown). However, as demonstrated in the simulated data, the frequency amplitude of the cleaned data (after artefact removal) is only ever slightly reduced at the frequency of stimulation, and never increased, and as such we can be confident that the increase in alpha amplitude found during the positive ramp sawtooth (relative to sham) is at worst a slight underestimate of the true alpha amplitude (if the true alpha amplitude were higher the effect would be more significant). Variations on the template subtraction method which overcome this limitation by creating the template from non-regular or pulsed oscillations (in a calibration phase prior to the regular tACS) are being investigated and will be discussed in future studies.

None of the stimulation conditions showed a significant effect on alpha power in the five minutes post-stimulation relative to the five minutes pre-stimulation. While other studies have found a significant after-effect of tACS on alpha power there are a number of differences in the experimental design which may explain why the current study did not show such an effect. Firstly we stimulated at 10Hz rather than adjusting the frequency of the stimulation to the individual alpha frequency of the participant as other studies have done (Neuling et al., 2013; Zaehle et al., 2010). Secondly, we only stimulated for ten minutes whereas other studies showing an after effect have applied stimulation for twenty minutes (Helfrich et al., 2014; Neuling et al., 2013). Zaehle et al. (2010) found an after-effect after 10 minutes of stimulation but with stimulation at individual alpha frequency and a different electrode montage to the one used here. Helfrich et al. (2014) used stimulation at 10Hz but stimulated for twenty minutes. This would imply that the sustained increase in alpha power after stimulation is dependent on either the stimulation frequency matching the individual's individual alpha frequency and/or stimulation with a duration of more than ten minutes.

Blinding is an on-going problem for all transcranial electrical stimulation research. As stated in the results, 17 of the 30 participants reported that they felt the stimulation for under a minute in all conditions, indicating that they were successfully shamed. However, the problem remains that some individuals are more sensitive to the sensation of tACS and were not successfully shammed. Other studies (Zaehle et al. 2010; Neuling et al. 2013) have adjusted the current intensity to the threshold of skin sensation for each individual rather than using a fixed current intensity. Adjusting the current intensity to each individual's threshold of

skin sensation is problematic because of the large variation in sensitivity to tACS across participants, as demonstrated by the wide variety of reports of sensation in the current study; different current intensities should not be compared as they may be having different effects on the cortex. This is especially important when considering the results of Moliadze et al. (2012) who showed that tACS can inhibit cortical excitability at low intensity and switch to excitation when the intensity is increased. There is no reason why the sensitivity of the scalp would correlate with the effect of the tACS on the cortex; therefore it is better to keep the intensity constant and control for sensation in some other way such as a control site or different stimulation parameters. Importantly, we found no difference between the sensation of positive and negative ramp sawtooth waves, as these were the two conditions we were comparing. Our results show a significant difference between positive ramp sawtooth stimulation and no stimulation, and no significant difference between negative ramp sawtooth and no stimulation. So in this context the sham condition can be considered a baseline condition. This finding may prove useful for future research because the effect of positive and negative ramp sawtooth waves is different, but the sensation is identical, and could therefore serve as a better control condition in future studies as the frequency, current density and skin sensations are identical for the two waveforms (albeit still requiring a baseline condition).

### *Conclusion*

The ability to stimulate with waveforms other than sinusoidal is an important addition to modern tACS stimulators, both because sinusoidal waveforms may not be optimal for entraining neural oscillations and because more can be learnt about the underlying mechanisms of transcranial electrical stimulation by systematically varying parameters such as the gradient of the electrical current. This preliminary investigation demonstrates that enhancement of alpha oscillations can be observed during positive ramp sawtooth stimulation, that the sawtooth artefact can be removed from single electrodes, and that sawtooth waves are not significantly different to sinusoidal stimulation in terms of side effects. Additionally, our results imply that current direction and gradient are important factors to consider in the design of tACS protocols. Further studies are needed to tell if this effect is frequency specific as well as if other waveforms, such as square wave, could also be useful variants of tACS.

# **Lateralized EEG responses during visually induced vection from visual flicker in the alpha range and subsequent modulation using frequency matched tACS.**

## **4.1. Abstract**

Self-motion perception is a key aspect of higher vestibular processing which has been suggested to rely upon hemispheric lateralization of cortical function and alpha-band oscillations. Here we investigated the role of alpha oscillations, and their lateralization, in the illusory sense of self-movement that can be caused by large optic flow stimuli (vection). Visual stimuli that flickered at alpha-frequency (approx. 10 Hz) were used in order to produce steady state visually evoked potentials (SSVEPs), a robust EEG measure which allows probing the functional importance of underlying frequencies. Differential lateralization of the alpha SSVEP response was found during vection compared to a matched random motion control condition. To test for a causal role of the right hemisphere in producing this lateralization effect, the frequency-specificity of the underlying neural generators was explored by applying transcranial alternating current stimulation (tACS) over the right hemisphere simultaneously with SSVEP recording, using a novel artefact removal strategy for combined tACS-EEG. During tACS, the alpha lateralization effect of vection was reduced and SSVEP amplitudes were enhanced. Subsequent control experiments showed the effect of tACS requires the flicker frequency and tACS frequency to be closely matched and tACS to be of sufficient amplitude. Taken together, these results support a role for lateralization of alpha-oscillations in self-motion processing. The combination of SSVEPs and tACS is a promising method both for future investigation into the role of neural oscillations in multi-sensory integration and attention, as well as for optimizing tACS as a research tool or clinical intervention.

## 4.2. Introduction

The unified perception of body position and self-motion requires the integration of information from the vestibular system with visual and somatosensory input; this is thought to occur via lateralization driven by the non-dominant hemisphere (Dieterich & Brandt, 2018; Dieterich & Brandt, 2015; Arshad, 2017). Vection, the illusion of self-motion, can be induced by presenting visual optic-flow and involves a temporary mismatch between vestibular and visual information. EEG studies (Palmisano et al., 2015) have found an increase in alpha activity during optic flow (peaking 14 seconds after stimulus onset) which correlated with stronger vection ratings (Palmisano, Barry, De Blasio, & Fogarty, 2016), although other studies have found alpha de-synchronization from optic flow for short (1 second) display times (Vilhelmsen, van der Weel, & van der Meer, 2015). Research into visual responses using EEG and actual body movement has found a suppression of alpha power (Gale et al., 2016; Gutteling & Medendorp, 2016; Ehinger et al., 2014). A common conclusion among these studies is that alpha power modulations are related to vestibular processing and self-motion estimates. These results are broadly in line with the hypothesis that coherence of oscillatory neural signals is critical for multisensory perception (Senkowski et al., 2008, Keil & Senkowski, 2018) and a general role for alpha-band oscillations in suppression and selection of attention and the ability to be consciously oriented in time and space (Klimesch, 2012).

If a modulation of alpha oscillations is critical for binding visual-vestibular information into a conscious percept, and the vestibular dominance in the right hemisphere in right handers is mediated by alpha oscillations, this prompts the current hypothesis that a lateralized response in the alpha band would result from the incongruent combination of visual and vestibular cues during vection, relative to moving stimuli which are inconsistent with self-movement where there is no mismatch with the vestibular sense.

Observing neuronal oscillations in response to various stimuli implies a functional role but this evidence is only correlational: To demonstrate a causal effect of neural oscillations, and to develop frequency targeted clinical interventions, direct manipulation of the oscillations is required (Herrmann, Strüber, Helfrich, & Engel, 2016). Here cortical activity in response to vection induced by optic flow was investigated by combining two techniques which both modulate neuronal oscillations in a frequency specific manner: Steady state visually evoked potentials (SSVEPs) and transcranial alternating current stimulation (tACS).

SSVEP research paradigms (Norcia, et al., 2015) typically involve various elements on a display flickering at one or more frequencies which can be measured in the EEG signal. SSVEPs can be seen in the EEG at frequencies ranging from 1-100 Hz, but show higher

amplitudes in resonant frequency bands (alpha, beta, gamma etc.) due to an interaction with the preferred frequency of endogenous neural oscillations in the cortex (Herrmann, 2001).

Transcranial alternating current stimulation (tACS) involves stimulating the cortex with a weak current via electrodes attached to the scalp at frequencies matching functionally relevant neural oscillations to entrain or modulate on-going brain activity. An increasing number of studies have demonstrated frequency specific effects of tACS in humans (e.g. Feurra et al., 2011; Wach et al., 2013), and these effects are thought to be due to the frequency of the stimulation being close enough to the oscillating neural activity that entrainment can occur (Herrmann, Rach, Neuling, & Strüber, 2013).

A combination of tACS and SSVEPs could potentially be a particularly useful experimental paradigm as the frequency and phase of the evoked neural oscillations can be precisely controlled and thus targeted with greater efficacy (Ruhnau, Keitel, Lithari, Weisz, & Neuling, 2016; Chai, Sheng, Bandettini, & Gao, 2018). In the current study two visual stimuli (optic flow and random dot movement) were used, matched for low level visual properties as a cognitive control to investigate any state specific effects of tACS.

The goals of the current study were, firstly, to investigate the potential of SSVEPs to probe cortical visual-vestibular interactions and hemispheric lateralization during vection, secondly, to demonstrate the ability of tACS to modulate this oscillatory response, and thirdly, to find a reliable effect of tACS on frequency matched SSVEPs with the aim of optimizing stimulation protocols in future research. To this end, a series of control experiments were conducted to demonstrate that tACS at nearby flanker frequencies, or at low amplitudes, does not have a significant effect on SSVEPs.

### **4.3. Methods**

#### *Overview*

SSVEPs were utilized to probe the response of the cortex to optic flow, in particular the relative responses of left and right hemispheres in the alpha range to test for hemispheric dominance of visual vestibular interaction. To target the role of alpha band oscillations in the right hemisphere tACS was applied with a right lateralized montage to healthy right handed participants. Participants were shown flickering full field optic flow stimuli, and a control condition of random dot movement, whilst standing, and were asked to report feelings of vection whilst EEG was recorded online, both during stimulation and in a baseline condition. Three separate experiments were carried out, each with an identical baseline condition and an identical condition in which tACS was matched closely to the flicker frequency. In addition



each experiment also contained two control conditions: experiment one contained tACS frequency controls, experiment two, tACS amplitude controls, and experiment three was a flicker frequency control.

### *Participants*

The first two experiments consisted of two sets of 10 participants. To investigate the test-retest reliability of any effects, the first ten who replied to invitation were tested again in a third experiment which then used 4 participants from experiment one, and 6 from experiment two. Participants had a mean age of 25 in experiment one (range 22-28, SD. 2.4) and 28 in experiment two (range 24 -33, SD 3.7). All participants self-reported as right handed and were tested on the Edinburgh handedness inventory (100 = fully right handed, -100 = left handed, 0 = ambidextrous); mean score was 86 (range 54-100, SD. 14.6) for experiment one and 74.5 (range 33-100, SD. 30.6) for experiment two.

The study was approved by the local ethics committee (LMU Medical Faculty). All participants had the experimental procedure explained to them, gave signed informed consent and were free to withdraw from the experiment at any time. One participant asked to withdraw from the experiment because the optic-flow stimulus was causing motion sickness; this subject was not included in the final analysis and was replaced by another.

### *Stimuli*

Stimuli consisted of two movies: optic flow dot pattern and a random dot motion control. Movies were created in MATLAB (The MathWorks Inc., Natick, MA, USA) using the Psychtoolbox extension. Stimuli consisted of white dots on a black background with a central fixation cross. Optic flow movies were created by first assigning a random position in 3D space to a large number of dots, the correct size and position of each dot from the perspective of the observer was calculated and converted to a screen position for each frame of the movie. The position of the observer was moved through space; this recreated the key features of true optic flow: object looming, acceleration towards the peripheral of the field of view, motion parallax etc. The random control movie consisted of the same number of dots as were on the screen in any one frame of the optic flow condition, the size of the dots was kept constant such that the number of white pixels on the screen (and therefore the total luminance) were on average the same as the optic flow movie. The dot motion was in a random direction for each dot and the speed was set to the average screen speed of the dots in the optic flow movie (approx. 15 degrees per second). In addition to the central fixation

cross a grey circle of approximately two degrees visual angle was in the centre of the screen during each movie to occlude any dots moving across the fixation point (2 degrees approximately corresponds to the fovea).

To evoke SSVEPs a flicker was introduced to the movies by darkening the dots (to middle grey) for two consecutive frames out of every six frames; this value was chosen to minimize the saliency of the flicker while still evoking a reliable SSVEP. If the refresh rate of the projector were exactly 60 Hz this would result in a 10 Hz flicker; however most displays do not update at exactly 60 Hz, in our case the true refresh rate (as measured with Psychtoolbox and external triggers sent with each frame) was approximately 59.89 Hz giving a flicker of approximately 9.9 Hz. We used this fact in our experimental design as we wanted the visual flicker to slowly drift in and out of phase with the 10 Hz tACS such that over the course of the experiment all relative phases were present. The fixation cross and central circle did not flicker.

### *Experimental design*

Participants were standing for the duration of the experiment; this was done to maximize the feeling of vection and to minimize any tactile clues that would be present if sitting. Viewing distance was one meter in front of a projection screen with the head supported by a chinrest. The display was projected (LCD projector, Epson) onto the screen using a mirror to increase the size of the display (see figure 10). This was done to allow the size of the display to be 90 degrees of visual angle in the horizontal plane and approximately 73 degrees in the vertical plane. The experiment was performed in a darkened room with the projector being the only source of light. Participants were instructed to fixate on the central cross throughout.

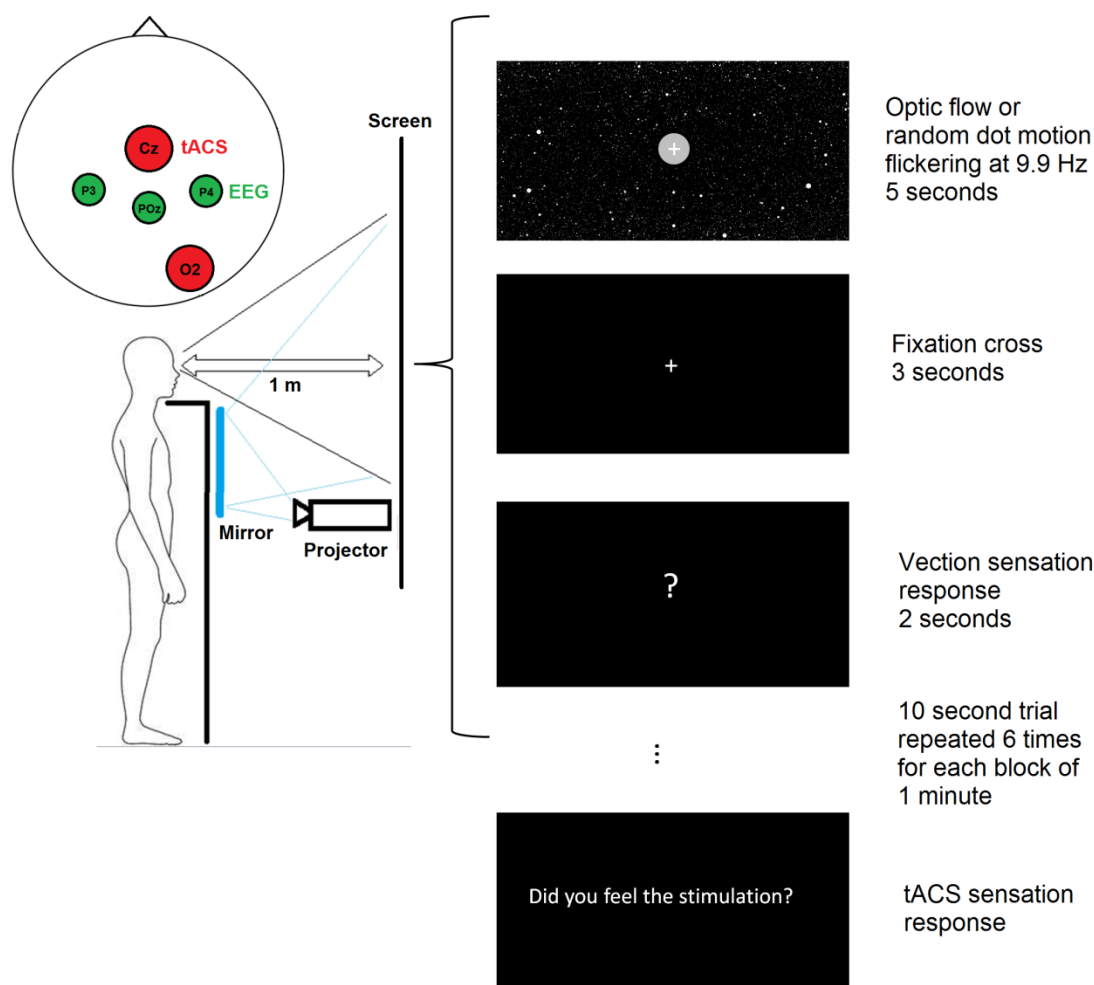


Figure 10: Experimental setup and procedure.

In each experiment participants were shown the stimuli in 24 blocks; each block contained six movies, three of optic flow, three of random dot motion, in randomized order. Each movie was five seconds long, followed by three seconds of a blank screen with a fixation cross, followed by a question mark prompting the subject to report their sensation of vection. Participants had two seconds to respond making a ten second trial, which was repeated six times to make a one minute block. The 24 blocks were randomly allocated into one of four stimulation conditions giving six blocks for each condition. Responses were manual button presses using one of four buttons on a custom-built response box, corresponding to No vection, weak vection, moderate vection and strong vection. At the end of each one minute block, participants were asked if they felt the tACS stimulation and if so how strong the sensation was compared to a short period of stimulation given at the beginning of the experiment (2 mA peak to peak), participants responded with the same four buttons indicating: No sensation, weaker/faint sensation, same as when they first felt the stimulation, stronger sensation. This was done to compare the sensations across conditions and to track how the sensation of tACS diminishes over time.

Participants were allowed to take short rests between blocks if requested. At the end of the experiment the participants filled out an additional questionnaire reporting their overall feeling of vection during the experiment for the two conditions (as a percentage from 0% to 100%, with 100% indicating strong vection, i.e. “it felt as if I was really moving” and 0% indicating no feeling of vection). In addition, participants were asked about any feeling of motion sickness, any adverse effects of the stimulation including any phosphenes.

## TACS

Electrical stimulation was applied with two circular silicon electrodes 4cm in diameter each, placed at positions Cz and O2 (Neuroconn Multi-channel stimulator, Munich, Germany). Cz and midline electrode Oz (not right hemisphere O2) have been used in a number of tACS experiments as modelling studies indicated that this montage is optimal for current reaching parietal lobes (Neuling et al., 2012). In the current study we placed the occipital electrode at O2 to bias the current flow towards the right hemisphere. Stimulating electrodes were attached using a conductive paste and the impedance was measured to insure it was below at least 10 k Ohms.

Each experiment consisted of four stimulation conditions; the order was randomized in all experiments. For the first experiment the four conditions were: tACS at 10 Hz, 8.3 Hz and 12.5 Hz (all with 1 mA amplitude, 2 mA peak to peak) and a baseline condition where the stimulation was switched off (no tACS). The second experiment (amplitude control) used three different amplitudes: 0.1 mA (0.2 mA peak to peak), 0.5 (1 mA peak to peak), 1 mA (2 mA peak to peak), all at 10 Hz, and a baseline condition. The third experiment consisted of two baseline conditions, one with the visual stimulus flickering at 9.9 Hz (as in the other experiments) and one with an approximately 8.57 Hz flicker (achieved by darkening two frames out of every 7), and two tACS conditions consisting of the two flicker frequencies with 10 Hz tACS at 1 mA (2 mA peak to peak). This control experiment was intended to both test if there is an interaction between optic-flow/random-movement and flicker frequency being closer or further from typical alpha frequency (10 Hz), as well a control to demonstrate that removing a 10 Hz tACS artefact does not corrupt SSSVEPs at other frequencies.

In a recent study, we applied tACS using various “sawtooth” waves in addition to the traditional sinusoidal waveform (Dowsett & Herrmann, 2016); waveforms such as square waves and sawtooth waves, which contain sudden transitions in current, may be more effective at influencing or entraining neuronal oscillations, but they have an additional advantage in that the distinct properties of sawtooth waves, i.e., consisting of straight lines with a steep transition, do not occur in nature and as such are more easily distinguishable

from neural activity. This makes removing the artefact from the EEG recording simpler (see below). As positive ramp sawtooth waves (and not negative ramp sawtooth) were previously found to enhance alpha oscillations during stimulation (Dowsett & Herrmann, 2016) this waveform was chosen for the current experiment.

### *EEG*

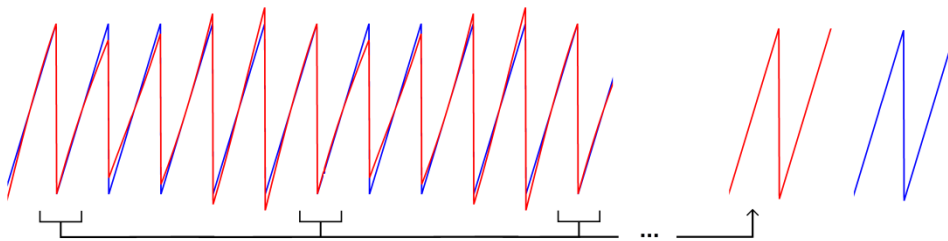
EEG was recorded from three electrodes at positions P3, POz and P4 on the 10/20 system (Ag/AgCl electrodes, BrainCap, Brain Products, Munich, Germany). These electrode positions are at the approximate midline between the stimulating electrodes where the stimulation artefact is smallest and unlikely to reach the limits of the range of the EEG amplifier and saturate the signal. EEG was amplified using a BrainAmp DC amplifier (Brain Products, Munich, Germany). Impedance of the EEG electrodes was kept below 5 k ohms and was recorded with a sampling rate of 5000 Hz. The EEG was recorded with the reference on the right ear lobe, and re-referenced offline to the average of two electrodes on each ear. The ground electrode was positioned on the forehead at electrode position Fpz.

### *Artefact removal*

The electrical artefact introduced to the EEG recording during stimulation can be several orders of magnitude larger than signals from on-going neural oscillations and needs to be removed before the data can be analysed. The majority of simultaneous tACS and EEG/MEG studies to date have removed the artefact with either beamforming (Neuling et al., 2015) or a combination of template subtraction and principle components analysis (Helfrich et al., 2014). However, each of these methods carry their own advantages and disadvantages and some researchers have documented how modulations in the size of the artefact due to heartbeat and breathing can result in residual artefacts which can easily be confused with entrained neural activity (Noury, Hipp, & Siegel, 2016; Noury & Siegel, 2018).

Here we present a modified “adaptive template subtraction” method which improves on the artefact removal in previous studies. Data was first segmented into 121ms segments time-locked to 10ms before the onset of each flash of the visual flicker. This length of time was chosen so that every segment during the tACS conditions contained at least one full cycle for all stimulation frequencies (8.3 Hz tACS has a period of 120ms). For each stimulation block, the tACS was on for the entire time and as such the artefact was present during the flickering stimulus and the inter trial interval (ITI). For each flicker segment containing a tACS artefact, a template was constructed by averaging matching segments from the ITIs from the same

stimulation block. This ensured that the SSVEP would not be included in the template and would not be subtracted. Previous template subtraction approaches have used a simple sliding average approach (Helfrich et al., 2014); however, due to changes in the size of the artefact an incorrectly sized template can easily result in a residual artefact. The stimulator always delivers a constant current and as such whenever the impedance changes the voltage will adapt and the size of the artefact in the EEG will change; changes in impedance can be due to a gradual drift in impedance from the conductive paste warming, participant movement or sweating as well as changes due to heartbeat and breathing (Noury et al., 2016). As such, rather than blindly creating a template, segments were selected which, when averaged, best matched the sawtooth artefact of the segment to be cleaned (figure 11).



*Figure 11: Illustration of the adaptive template selection procedure: In ideal cases the sawtooth artefact would be constant amplitude (blue); however in reality the artefact changes in size over time (red, amplitude modulation not to scale). Only segments where the sudden jump of the sawtooth closely matches the segment to be cleaned are averaged to make the template.*

Specifically, the time point of the steep transition from anode to cathode in the sawtooth wave was identified and the first derivative (i.e. the gradient) of the cleaned segment was minimized for 2ms around this time point. A steep gradient at this time point would be the clearest evidence of a residual artefact; sinusoidal tACS would not contain any such steep gradients and it is much harder to identify a residual artefact. Importantly, only this time range was used and not the entire segment which could potentially lead to over-fitting the template to the evoked response, i.e. if the template were optimized to result in the smallest overall amplitude across the entire segment then neural activity could also be included in the template and subtracted. For each segment to be cleaned, first the 20 best segments from the surrounding ITIs were selected (i.e. segments that when subtracted from the segment to be cleaned gave the lowest value in the first derivative at the critical time point). Next, 100 additional segments from the surrounding ITI were considered in turn, included in the average template and subtracted from the segment to be cleaned: if there was an improvement then the segment was included in the optimal “running average” template then

the next potential segment was considered. This was repeated for up to 100 segments; when more than 100 segments are averaged to create a template very little additional improvement can be seen. Once the optimal template had been found, this was then subtracted from the original raw segment to produce the final cleaned segment of data. This procedure was performed separately for each and every segment of data.

This method has a number of advantages: firstly, as only a short segment is being cleaned at any one time it is more likely that a correctly matching segment can be found: if the artefact is being removed from longer periods (e.g. one second) the artefact might have changed in size far more in that time and it is less likely that a correctly matching template can be constructed. Secondly, as the template matching algorithm is only considering short segments at any one time it is “blind” as to the frequency of the artefact, the only input is 121ms of data, and as such the artefact removal is no less likely to be successful across the 8.3 Hz, 10 Hz and 12.5 Hz conditions. This overcomes some of the problems that can arise from removing the artefact from longer segments such as “side band” artefacts in the FFT where an amplitude modulated artefact can appear as two peaks at neighbouring frequencies (Noury et al., 2016).

### *EEG Data analysis*

After tACS artefact removal, any segments containing eye-blinks or movement artefacts were rejected (range greater than 50  $\mu$ V in EOG channel or 200  $\mu$ V in the segment itself), and the remaining segments of data were averaged to create an SSVEP. At least 500 segments were averaged for each SSVEP. Each SSVEP was then low-pass filtered at 30 Hz to remove high frequency and 50 Hz line noise (4<sup>th</sup> order Butterworth filter). An additional 10ms were removed from each end of the segment to give one cycle of the SSVEP and to remove edge artefacts from the filter. For each SSVEP the peak-to-peak amplitude was taken as the dependent variable. It is common in SSVEP experiments to perform a frequency transform (FFT) on a segment of the data to describe the amplitude of the evoked oscillation. However, here we chose not to do this for the main analysis: firstly because our particular artefact removal method works optimally for shorter segments in which an FFT would have very low resolution, and secondly because the evoked oscillations are non-sinusoidal and would show higher harmonics which would be distributed across the FFT spectrum (in most cases a 10 Hz oscillation was evoked from the flicker but in some participants the SSVEPs were a 20 Hz oscillation). Here the SSVEP was treated more like a traditional event related potential (ERP) and the peak to peak amplitude allowed the total size to be captured in a

single number regardless of waveform shape (see figures 20 and 21 for examples of individual data).

In addition to the main analysis, we determined the individual alpha frequency of each participant. To do this, the data from the inter-trial intervals in the baseline (no stimulation) condition was split into two second segments centred on each ITI, segments containing eye blinks or movement artefacts were rejected as before, an FFT was performed on each and the resulting spectra were averaged for each participant. The individual alpha frequency was defined as the peak of the averaged spectra between 8 and 14 Hz.

### *Statistical analysis*

For the analysis of the (non-parametric) behavioural data, a Friedman test was performed on the median responses to each condition and followed up with Wilcoxon signed rank tests.

For the analysis of the EEG data, the SSVEPs were first analysed in the baseline condition and the 10 Hz 1mA tACS condition for experiments one and two combined as these conditions were identical. To investigate the lateralization of evoked potentials across hemispheres the ratios of the peak-to-peak amplitude from P3 and P4 were compared (P3/P4) in addition to the peak-to-peak amplitudes. A 2 x 2 repeated measures ANOVA was performed on the ratio values with visual stimulus (optic flow and random movement) and stimulation (no stimulation and 10 Hz 1mA tACS) as factors. A 3 x 2 x 2 repeated measures ANOVA was performed on the peak-to-peak amplitudes with electrode (P3, POz, and P4), visual stimulus and stimulation as factors. Post-hoc t-tests with Bonferroni correction were used throughout where appropriate.

Next, the peak-to-peak amplitudes of the SSVEPs, as well as the P3/P4 ratios, were compared separately for each experiment.

To further investigate the role of the relative phase of the tACS and the SSVEP, separate evoked potentials for ten phase bins were created for each participant. The ten phase bins were ten-millisecond periods determined by the time between the onset of each visual flash and the steep transition of the sawtooth wave, i.e. the first bin being 0-10ms, the second 10-20ms etc. Thirty segments of cleaned data were averaged for each bin to insure no one bin had a significantly larger number of trials. These segments were selected at random from all artefact free segments from the entire experiment to ensure no condition contained segments preferentially from earlier or later in the experimental session.



The 10 Hz tACS condition in the first experiment, the 1mA (10 Hz) condition in the second experiment and the 10 Hz tACS/9.9 Hz flicker condition in the third experiment all showed significant increases in the amplitude of the evoked potentials at certain phases in most participants, albeit at different phases for each participant. This indicates that the overall effect is driven by certain optimal relative phases of tACS and visual flicker (see figure 12). To quantify this phase-specific effect, an optimal phase bin was selected for each participant (the phase bin with the largest peak-to-peak amplitude) as well as a “least optimal” phase bin (the phase bin with the smallest peak-to-peak amplitude). The ratio of the amplitudes in the optimal compared to least optimal bin was taken as a measure of the amount of variability in the SSVEP that is due to the relative phase of the tACS and the visual flicker. For the baseline conditions, segments were assigned into one of 10 bins at random (again with 30 segments for each bin but selected at random from throughout the experiment). These “random bins” give an indication of how much variability would be expected if there was no effect of phase and the null hypothesis were true. For example, a ratio score of 3 in the baseline condition would indicate that the amount of variation expected if there is no effect of phase of tACS would be an optimal phase bin three times larger than the least optimal phase bin. This value was then compared to the ratios during stimulation with t-tests. Electrode POz was selected for the analysis of the tACS/flicker phase effect as this electrode consistently gave the largest SSVEPs and data from both visual stimulus conditions were combined to maximize the number of phase bins for which 30 artefact free segments could be found.

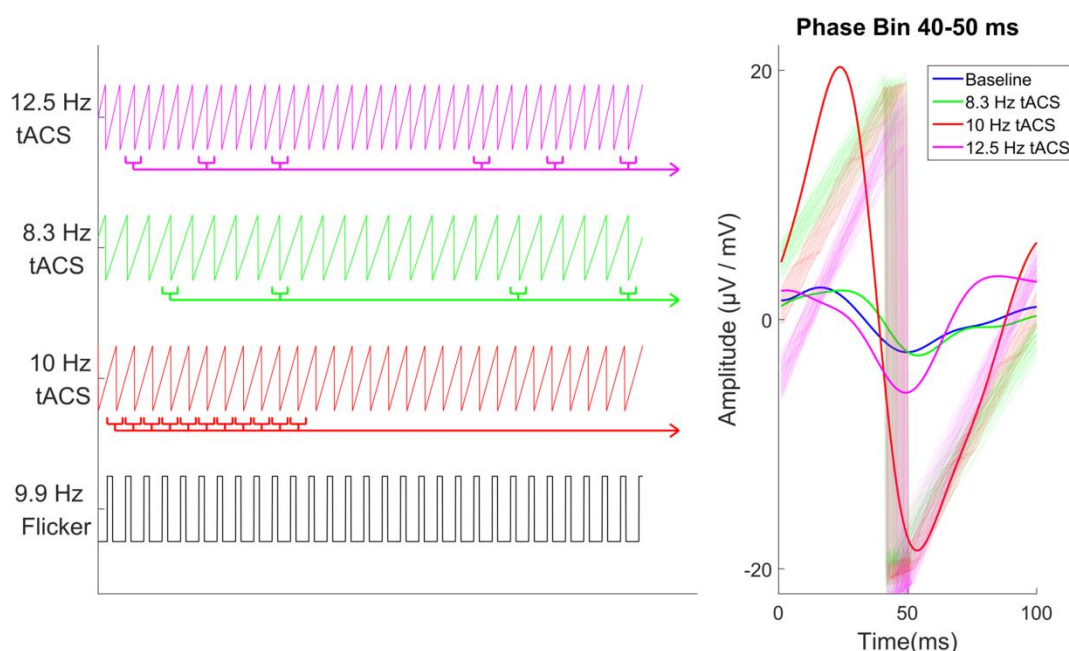


Figure 12: Right: Example SSVEPs from one participant from the optimal phase bin from electrode POz. This phase bin corresponds to the sudden jump of the sawtooth stimulation occurring between 40 and 50 ms after the onset of each flash of the visual flicker. SSVEPs are shown in thick lines (y-axis in  $\mu\text{V}$ ); all corresponding raw segments before artefact removal and averaging are shown with thin lines (y-axis in mV, i.e. 1000 times larger). SSVEP during 10 Hz tACS is significantly enhanced despite the similarity of the raw segments which would be no more likely to leave a residual artefact in any condition. Left: illustration of how segments corresponding to this phase bin would be distributed across a few seconds; tACS of different frequencies drifts in and out of phase with the 9.9 Hz visual flicker. (Note: for analysis 30 segments were averaged to create each Phase bin SSVEP, randomly selected from throughout the experiment).

## 4.4. Results

### Behavioural data

The Friedman test on the median responses to each condition showed a significant effect of condition in all three experiments ( $p < 0.01$  for all). Post hoc Wilcoxon signed rank tests showed this result to be driven by higher feelings of vection during optic flow compared to random dot movement in all conditions ( $p < 0.05$  for all cases, significant after Bonferroni correction for baseline and 1 mA tACS conditions in experiment two and for the baseline condition in experiment three) and no significant effect of tACS or flicker frequency ( $p > 0.5$  between all stimulation conditions in all experiments).

*EEG lateralization: Combined data from experiments one and two*

Data from experiments one and two were first combined giving 20 participants. P3/P4 Ratio were calculated for optic-flow and random dot movement conditions (figure 13, right). The 2 x 2 ANOVA on the ratio values showed a significant effect ofvection ( $p = 0.01$ ), no overall significant effect of tACS ( $p = 0.6$ ) and no significant interactions. Paired sample t-tests showed a significant effect of visual stimulus in the baseline condition ( $p = 0.0096$ ). There was no significant difference between optic-flow and random motion during 10 Hz 1 mA tACS ( $p = 0.31$ ) indicating that the effect was removed by stimulation. Optic-flow was still more left lateralized than random dot motion during tACS; the P3/P4 lateralization in baseline and tACS for the random motion was very similar, indicating that the effect of the tACS was mainly stimuli specific to optic-flow and had the effect of shifting the natural lateralization to the right.

To explore this P3/P4 lateralization in more detail, a separate analysis was run on the SSVEP peak-to-peak amplitudes from each electrode (figure 13, left). Experiments one and two combined showed a highly significant effect of tACS ( $p < 0.0001$ ), no significant effect of visual stimulus (although marginal if non-corrected  $p = 0.055$ ), and a significant effect of electrode ( $p = 0.015$ ). Post-hoc t-tests show a significant increase in SSVEP amplitudes in the tACS condition compared to baseline for each of the electrodes separately ( $p < 0.001$  in all cases). Post-hoc t-tests between electrodes did not reveal any significant effect ( $p > 0.05$  in all cases); the significant effect in the ANOVA was largely driven by larger SSVEPs from electrode POz.

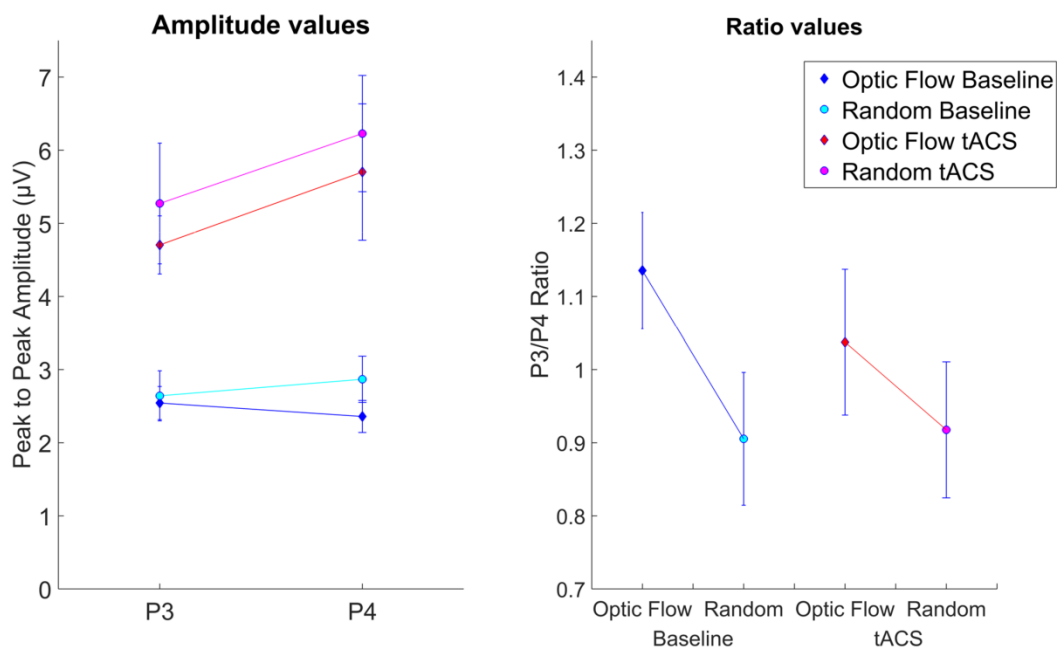


Figure 13: Mean peak-to-peak SSVEP amplitudes from electrodes P3 and P4 only (left) and mean P3/P4 ratio (right) for all 20 participants from experiments 1 and 2, for the baseline and 10 Hz 1 mA tACS conditions. Error bars indicate one standard error of the mean (SEM).

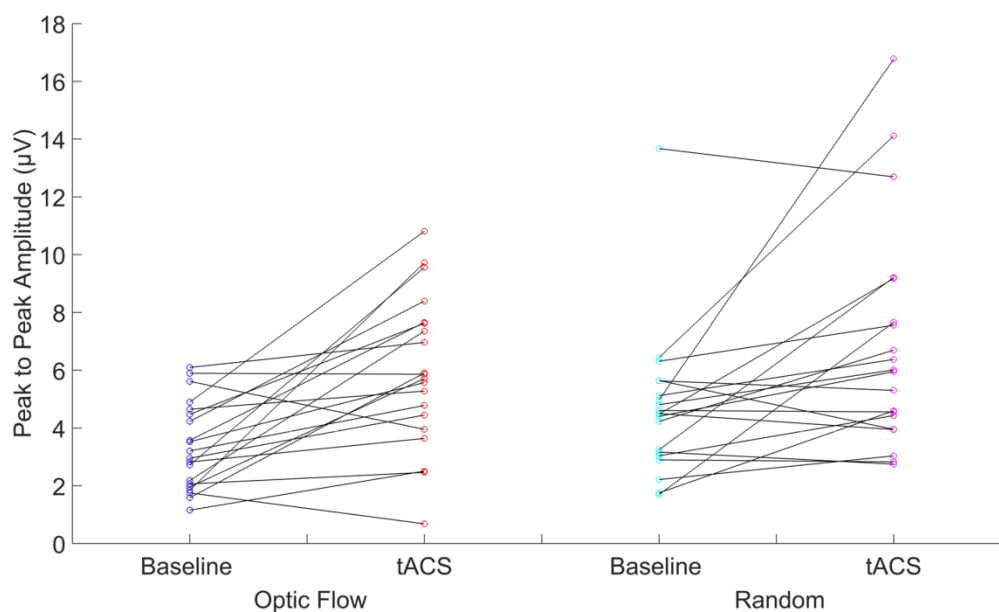


Figure 14: Individual SSVEP Peak-to-peak amplitudes from POz for all 20 participants from experiments 1 and 2. Lines indicate the change in amplitude between baseline and 10 Hz 1 mA tACS.

### Baseline data from all experiments

The same analysis was performed for the P3/P4 ratios from the baseline condition of each experiment separately. For experiments 2 and 3 the paired sample t-test showed a significant effect of P3/P4 lateralization (experiment two  $p = 0.003$ , experiment three  $p = 0.004$ ) as in the combined data from experiments 1 and 2. Experiment 1 did not reach significance with all subjects included ( $p = 0.3$ ) but this data included one obvious outlier for whom the change in P3/P4 ratio across conditions was completely different and more than two standard deviations from the mean ratio difference (shown in red, figure 15); with this outlier removed the paired sample t-test became significant for experiment 1 ( $p = 0.028$ ). Again, the average was left lateralization for optic flow and right lateralization when viewing random dot motion. This outlier was included in all other statistical tests as the participant was only an outlier for the P3/P4 ratio in the baseline condition and showed no unusual behavioural data (removal of this outlier would have made no difference to the outcome of any the other statistical test).

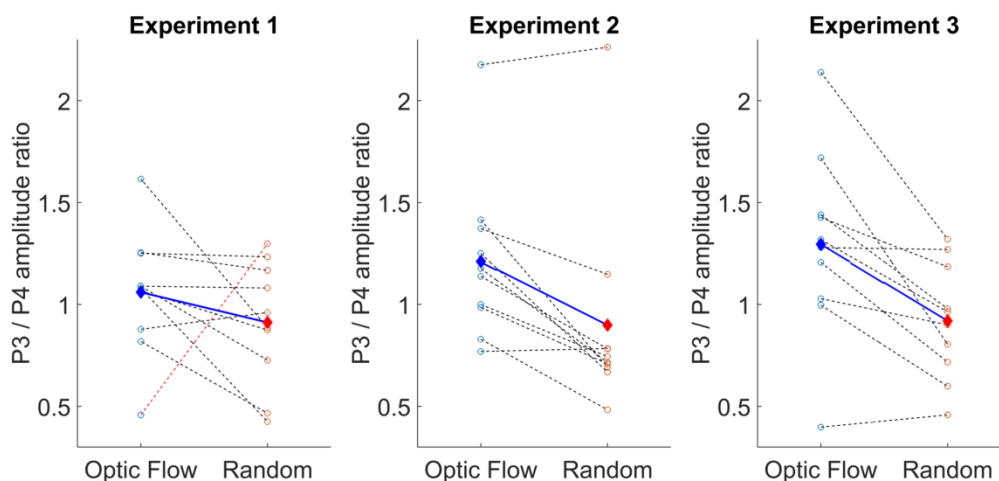


Figure 15: P3/P4 ratios from the baseline conditions from all three experiments separately. Circles and dashed lines show individual participants (significant outlier from experiment 1 shown in red), Diamonds indicate group means.

Next, EEG data from all conditions were analysed separately for each experiment.

### Experiment 1: Frequency control experiment.

A three-way repeated measures analysis of variance on the peak-to-peak amplitudes showed a highly significant effect of tACS ( $p < 0.0001$ ), a significant effect of visual stimulus ( $p = 0.024$ ) and a non-significant effect of electrode ( $p = 0.48$ ), and no significant interactions. Post-hoc t-tests showed a significant increase in SSVEP amplitudes in the 10 Hz tACS

condition compared to all other conditions for any one electrode ( $p < 0.001$  in all cases) and no significant differences between any other tACS conditions and baseline for any electrode or visual stimulus condition ( $p > 0.9$  in all cases). This demonstrates that the effect of the tACS is specific to conditions when the frequency closely matches the flicker (see figures 20 and 21 at the end of this chapter for examples of individual data).

The mean SSVEP amplitudes for the random dot patterns were larger than the for the optic flow condition for all electrodes and stimulation conditions, driving the significant effect of visual stimulus in the ANOVA, but this did not reach significance for any one electrode or stimulation condition.

The repeated measures ANOVA on the P3/P4 ratios did not show any significant effects in experiment 1, but see above outlier in the baseline condition. Visual inspection suggested that the direction of the lateralization was preserved across all conditions (P3 larger than P4) except for the 10 Hz tACS condition.

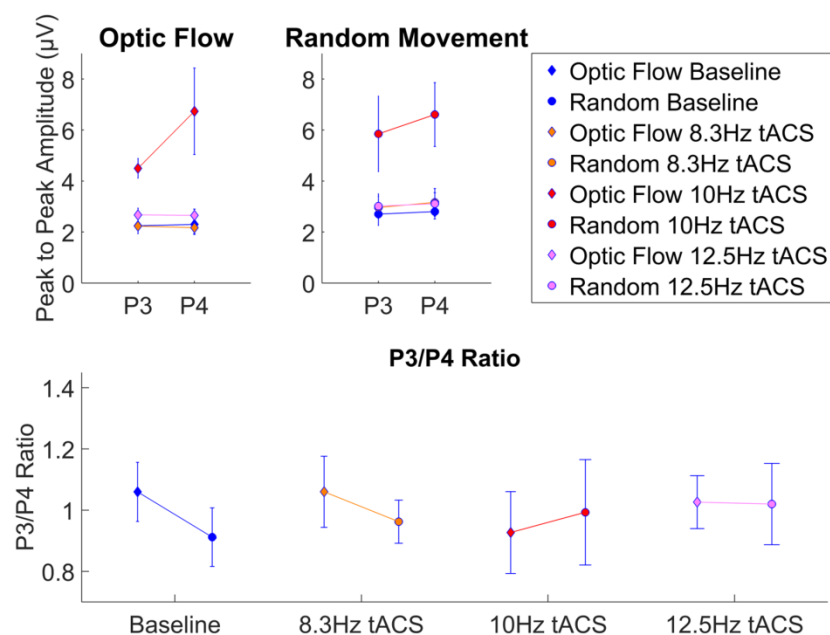


Figure 16: Mean peak-to-peak SSVEP amplitudes for experiment 1 for optic-flow condition (top left) and random dot motion (top right) and the P3/P4 ratios (bottom). Diamonds indicate optic flow, circles indicate random dot movement. Error bars show one SEM.

### Experiment 2: Amplitude control experiment

The three-way repeated measures ANOVA on the peak-to-peak amplitude for each condition showed a highly significant effect of tACS ( $p < 0.0001$ ) and a significant effect of electrode ( $p$

$< 0.0001$ ). Post-hoc t-tests showed a significant increase in SSVEP amplitudes in the 1 mA tACS condition compared to baseline and 0.1 mA tACS for any one electrode ( $p < 0.001$ ) and no significant differences between 0.5 mA and any other stimulation condition ( $p > 0.1$  in all cases). As with the combined results of experiments 1 and 2 the significant effect of electrode was driven by larger SSVEPs from electrode POz which were not individually significant after correction for multiple comparisons.

The repeated measures ANOVA on the P3/P4 ratios showed a significant effect of visual stimulus ( $p = 0.01$ ), a non-significant effect of tACS ( $p = 0.7$ ) and a significant interaction between visual stimulus and tACS ( $p = 0.04$ ). Post hoc t-tests showed a significant effect of visual stimulus in the baseline condition ( $p = 0.003$ ). The effect of visual stimulus in the 0.1 mA tACS condition trended towards significance ( $p = 0.1$ ) and all other stimulation conditions were non-significant after correction for multiple comparisons ( $p > 0.1$ ). Inspection of the data (Figure 17) showed the direction of the lateralization was preserved across all conditions (P3 larger than P4) except for the 0.5 mA condition.

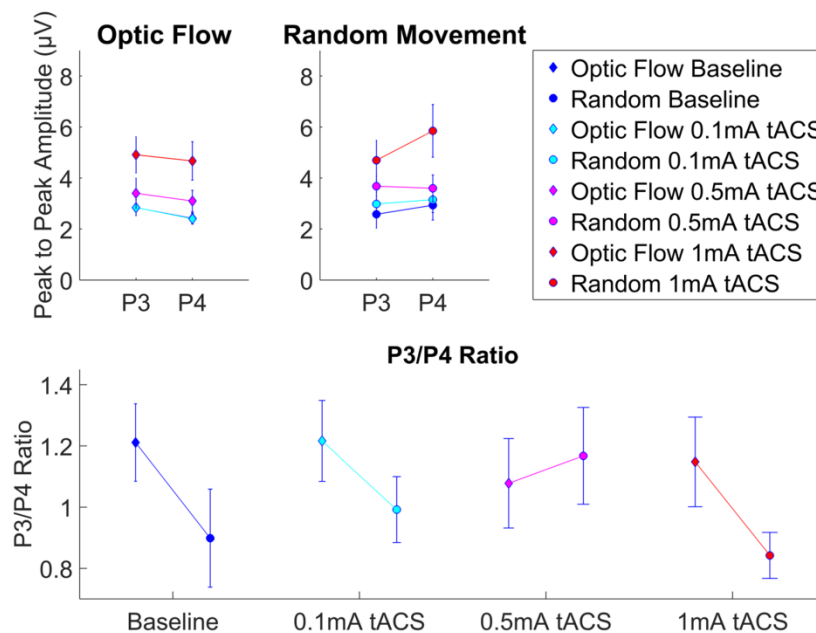


Figure 17: Mean peak-to-peak SSVEP amplitudes for experiment 2 for optic-flow condition (top left) and random dot motion (top right) and the P3/P4 ratios (bottom). Diamonds indicate optic flow, circles indicate random dot movement. Error bars show one SEM.

### Experiment 3: Flicker frequency control experiment

The repeated measures ANOVA showed a highly significant effect of electrode ( $p < 0.0001$ ), a non-significant effect of visual stimulus ( $p = 0.07$ ), a significant effect of flicker frequency ( $p = 0.04$ ) and a significant effect of tACS ( $p = 0.03$ ). In addition, there was a significant interaction between flicker frequency and tACS ( $p = 0.016$ ). Post hoc t-tests showed a significant difference between 9.9 Hz flicker with no stimulation and 9.9 Hz flicker with 10 Hz tACS ( $p < 0.001$ ) and no significant difference between 8.5 Hz flicker and 8.5 Hz flicker with 10 Hz tACS ( $p > 0.1$ ), therefore driving the interaction between flicker frequency and tACS.

The repeated measures ANOVA on the P3/P4 ratios showed a significant interaction between visual stimulus and tACS ( $p > 0.001$ ). Post hoc t-tests showed that this interaction was driven by a significant difference between optic-flow and random dot motion in the 9.9 Hz flicker baseline condition ( $p = 0.004$ ) and no significant difference for 9.9 Hz flicker and 10 Hz tACS ( $p = 0.6$ ) as in the combined results of experiments 1 and 2. There were no significant differences between visual stimulus conditions for any 8.5 Hz flicker conditions ( $p > 0.05$  for all). This demonstrated that the lateralization effect found in all experiments in the 9.9 Hz flicker does not hold when the flicker is 8.5 Hz. It is of note that FFT spectra of the baseline ITI showed that 19 out of 20 participants across all experiments had an individual alpha frequency of 10 Hz or higher; as such the specificity of the effect at 9.9 Hz could be explained as an interaction of the SSVEP with the on-going alpha band oscillations.

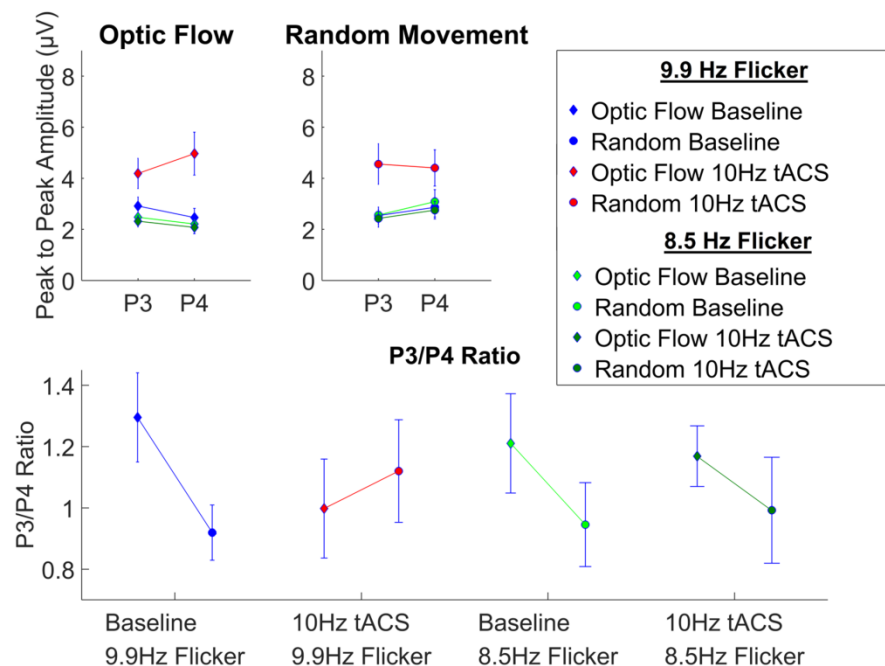


Figure 18: Mean peak-to-peak SSVEP amplitudes for experiment 3 for optic-flow condition (top left) and random dot motion (top right) and the P3/P4 ratios (bottom). Diamonds indicate optic flow, circles indicate random dot movement. Error bars show one SEM.



### Phase specific effects

For each experiment the optimal/worst phase bin ratio was compared to baseline for each condition with multiple paired sample t-tests. No differences reached significance for experiment 1, although baseline vs. 10 Hz tACS was close to significance ( $p = 0.07$ ). Descriptively, the values for the 8.3 Hz and 12.5 Hz tACS were very similar to baseline (see figure 19,  $p > 0.5$  for both) indicating no phase specific effect of tACS at flanker frequencies. For experiment 2, the 1 mA condition was significantly higher than baseline ( $p = 0.015$ ) indicating a phase specific effect of tACS. The 0.5 mA condition was close to significance but did not survive Bonferroni correction ( $p = 0.02$ ). The 0.1 mA condition was also very similar to baseline ( $p > 0.5$ ) demonstrating that the removal of a 10 Hz artefact does not bias the optimal/worst phase bin measure. For experiment 3 there was a significant difference between baseline 9.9 Hz flicker and 9.9 Hz flicker with 10 Hz tACS ( $p = 0.006$ ) again demonstrating that the overall effect of tACS was largely phase specific. The difference between 8.5 Hz flicker baseline and 8.5 Hz flicker with 10 Hz tACS was not significant after Bonferroni correction ( $p = 0.04$ ).

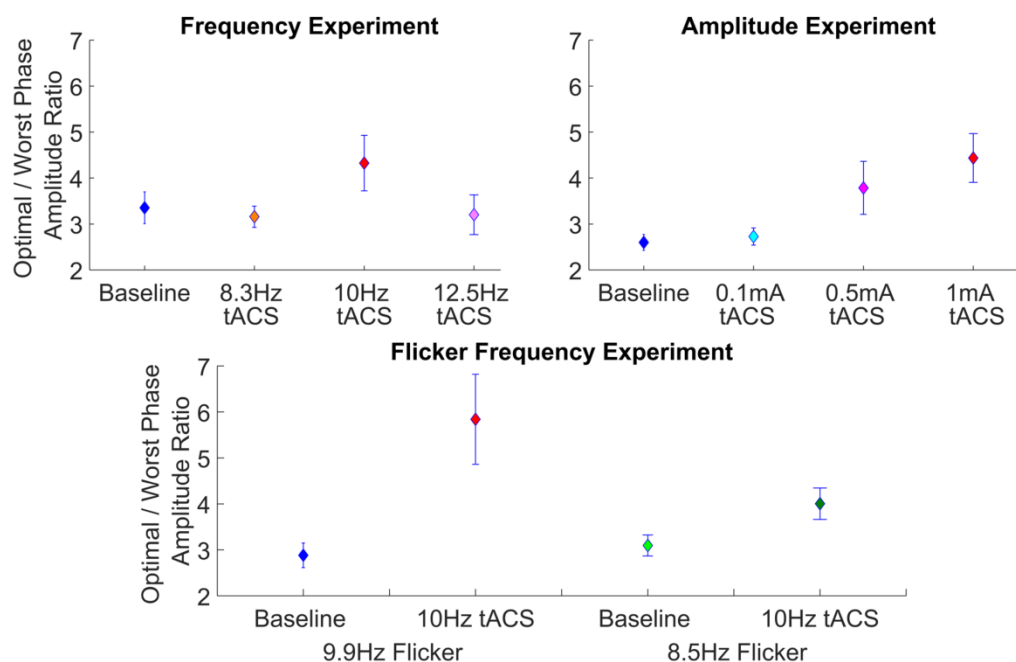


Figure 19: The ratio of optimal phase bin to worst phase bin for each experiment (electrode POz, both visual stimulus conditions combined). Values significantly higher than baseline indicate a phase specific interaction between tACS and SSVEPs only at certain optimal phase bins. Error bars show one SEM.

### *Individual Alpha Frequency*

The FFT spectra from the inter-trial intervals showed a mean frequency of 10.4 Hz (max. 11.5 Hz min 8.5 Hz) across all participants. It is of note that only one participant had an individual alpha frequency below 10 Hz, i.e. all others were between 10 and 11.5 Hz and therefore both above and quite close in frequency to the tACS and the visual flicker.

### *Post experiment questionnaire*

The average response to the overall feeling of vection across the entire experiment was 72.5% and 5.7% for optic flow and random dot movement respectively for experiment 1, 60.5% and 15% for experiment 2, and 55.7% and 18.5% for experiment 3.

In answer to the question “did you ever feel dizzy or disorientated?”: for experiment 1, 4/10 responded “yes” for the optic flow condition and 3/10 responded “yes” for the random dot motion, in experiment 2, 4/10 responded “yes” for the optic flow condition and 3/10 responded “yes” for the random dot motion, and in experiment 3, 3/10 responded “yes” for each condition (although not the same participants).

In response to the question of whether or not they saw phosphenes during the tACS (it was made clear that this was not the flickering screen but due to the stimulation when there was no visual flicker) only two participants from all 30 participants across all three experiments reported seeing phosphenes, and one of these reported that this was only at the beginning of the stimulation.

### *Test-retest*

A comparison was done between the P3/P4 ratios from the baseline conditions of the 10 participants who took part in experiment 3 and either experiments 1 or 2. A paired sample t-test showed the two groups were not significantly different ( $p = 0.83$ ), however, a pairwise correlation showed the two were not significantly correlated ( $r^2(8) = -0.44$ ,  $p = 0.1$ ).

Descriptively, the results were quite varied with 2 out of 10 participants showing ratios in experiment 3 within 10% of the first time they were tested, 6 out of ten were within 20%, and 2 participants showed ratios more than 50% different.

A comparison of the overall vection ratings for the optic flow condition from the 10 participants who took part in more than one experiment were equally varied ranging from a difference of 2% to a difference of 60%, with a mean absolute deviation of 28.7%. This

indicates that some subjects experienced different sensations of vection across different days, whereas others remained consistent.

#### *TACS sensation*

The median responses to the question of whether participants felt the tACS more or less than at the beginning of the experiment was calculated (0 = no sensation, 1 = less, 2 = same, 3 = more). For the first and third experiments the median scores were zero for Baseline, and one for all tACS conditions. For the second experiment: one for 1 mA tACS and zero for all other conditions. Therefore most participants felt the stimulation less as the experiment progressed. Wilcoxon signed rank tests showed no significant differences between any conditions ( $p > 0.1$  in all cases). These values were highly variable with some participants feeling nothing in both experiments, which explains the lack of a significant difference between the sensation in the baseline and 1 mA tACS conditions at the group level. Importantly the sensations were virtually identical between the various frequencies of tACS in experiment one so the significant effect of 10 Hz stimulation cannot be due to sensation.

### **4.5. Discussion**

In the present study it was shown that that optic-flow evokes lateralized responses compared to random dot motion when flickering in the alpha range and that this lateralized response can be modulated with frequency matched tACS to the right hemisphere.

For most individuals the SSVEPs shifted to being smaller in the right hemisphere and/or larger in the left hemisphere during optic-flow relative to random dot movement. Inspection of the SSVEP amplitudes indicated that this effect was largely driven by differences in the right hemisphere. The two conditions were matched for total luminance and were on average symmetrical in the vertical midline and as such these hemispheric differences cannot be explained by low level stimulus properties. As the optic flow stimulus reliably induced vection, and in some cases motion sickness, this EEG effect was a neural correlate of vection. This provides additional evidence for the lateralization of visual-vestibular function, and in particular in the alpha band.

The overall effect of frequency matched tACS applied to the right hemisphere (across 20 participants) was to nullify this lateralization effect. There was very little difference between the average P3/P4 ratio from the flickering random dot motion between the baseline and tACS (0.90 and 0.91 respectively); the lateralization effect becoming non-significant during

tACS was driven by a difference in the optic-flow condition (an average ratio of 1.135 in the baseline compared to 1.037 during tACS, see figure 3). Therefore, the effect of right hemisphere tACS on lateralization was specific to optic-flow, despite the amplitudes of the SSVEPs being significantly enhanced across all electrodes and in all visual conditions. A potential explanation for this is that the additional processing of incongruent optic-flow/vestibular information in the right hemisphere (as indicated by smaller SSVEPs in the baseline condition) resulted in a greater susceptibility to the driving force of the frequency matched tACS and the subsequent disruption of the lateralization ratio, whereas during random dot motion neither hemisphere is dominant and the lateralization ratio was preserved. Further insight as to why this might occur comes from a study which used simultaneous invasive recording and 10 Hz-pulsed electrical stimulation direct to the cortex of patients before epilepsy surgery (Alagapan et al., 2016). The authors found that the effect of the stimulation depended on the brain state of the participant: with large neural oscillations (eyes closed) the stimulation had little effect on the oscillation dynamics, with weaker oscillations (eyes open) the stimulation altered power at the natural frequency and in a task-engaged state the stimulation evoked a response matched to the stimulation frequency. This could explain why in the current study a weaker response to visual flicker in the right hemisphere during optic-flow (due to task-engagement of visual-vestibular conflict) was more easily enhanced by tACS to the right hemisphere, resulting in the disruption of the normal lateralization.

TACS consistently increased the amplitude of SSVEPs only when the frequency was closely matched and the amplitude was sufficiently high. All control conditions across all three experiments (except 0.5 mA tACS, see below) showed no effect of stimulation either in the peak-to-peak amplitude of the SSVEPs, in the P3/P4 ratio, or any phase specific amplitude modulations. Taken together this is good evidence that the most significant effects of tACS occur when the frequency closely matches the on-going neural activity and is of sufficient amplitude.

Progressively weaker alternating currents have been shown to be able to entrain neural activity as the stimulating frequency approaches the frequency of the neuronal oscillation, a relationship known as an Arnold Tongue; as such the maximal effect is predicted to be when the frequency of the neuronal oscillation and the stimulation are closely matched (Fröhlich, 2015). A number of tACS experiments have attempted to optimize the efficacy of stimulation by matching the frequency of the stimulation to individual alpha frequency (Zaehle, Rach, & Herrmann, 2010; Neuling, Rach, & Herrmann, 2013; Kasten & Herrmann, 2017). However, naturally occurring oscillations are not a precise frequency and can shift in phase and frequency over time and depending on task. Combining SSVEPs and tACS can provide a

solution as the frequency of the induced neuronal oscillation can be precisely controlled because it will always follow the driving flicker of the visual stimulus, this could significantly increase the efficacy of tACS, although strictly speaking there is no phase or frequency shift of the SSVEP as it is frequency locked to the visual flicker. Rather, what we observed here could be called “constructive interference” between the neural oscillation and the stimulation. This effect is present when the tACS and neural oscillation are close but not exactly matched (9.9 Hz flicker, 10 Hz tACS), demonstrating that neural and external oscillators do not have to be exactly the same frequency to show significant enhancement. This could help bypass some of the technical difficulties involved in stimulating and recording at exactly matched frequencies.

The artefact removal method used here has a number of advantages over other methods. The template subtraction algorithm is no more likely to leave a residual artefact in any one of the tACS frequency conditions; this is because the SSVEP is treated like a traditional ERP and only a short segment of data, approximately the length of one cycle of the stimulation, has the artefact removed at any one time. The 0.1 mA control condition showed no effect which excludes the possibility that removing an artefact at 10 Hz leaves a residual artefact, and the flicker frequency control (10 Hz stimulation with 8.5 Hz flicker) showed no effect on the amplitudes of the SSVEPs which excludes the possibility that creating a template from 10 Hz 1 mA stimulation leaves a residual artefact or somehow corrupts the data. Furthermore, the fact that the tACS and flicker were slightly different frequencies means that they drifted in and out of phase and all relative phases would be included, as such any small residual artefact that may have been left in the raw segments would not be phase locked to the flicker and would be greatly reduced in the process of averaging. As such, we can be confident that the effect is a genuine manipulation of neural activity. In addition to the increase in amplitude only the 10 Hz 1 mA tACS showed a phase specific modulation on the amplitudes of the SSVEP, indicating an interaction between two oscillators.

Other findings of interest include the effect of 10Hz tACS at 0.5 mA in experiment two: the amplitude of SSVEPs was enhanced, although not significantly, and in addition there was a reversal of the lateralization effect which can be seen in the significant difference between P3/P4 ratios across optic-flow and random dot conditions. We chose 1 mA (2 mA peak-to-peak) for the main stimulation condition because this intensity has shown effects in previous studies and stimulating at lower intensities was less likely to show an effect. However, the peak-to-peak amplitudes of the SSVEP were clearly enhanced in some cases by the 0.5 mA tACS. The wide spread of amplitudes during 0.5mA stimulation across participants (i.e. a high standard deviation) might indicate responders and non-responders; this intensity may have been above the threshold required to affect neural oscillations for some participants

and not for others. Another possibility is that the 0.5 mA tACS had a different effect to the 1 mA tACS (as implied by the reversal of the effect on the P3/P4 ratios) due to non-linear effects of stimulation. Moliadze and co-workers reported that high frequency tACS over the motor cortex led to increased cortical excitability with 1 mA, inhibition of cortical excitability at 0.4 mA and no effect with the intermediate 0.6 and 0.8 mA (Moliadze et al., 2012). It is possible that such non-linear effects may also occur in the current paradigm and future experiments should explore this possibility by testing multiple intensities over multiple sessions as it is likely that different individuals have different excitability thresholds.

Another interesting finding was that the lateralization effect could be seen in the baseline condition with 9.9 Hz flicker in experiment 3, but not in the 8.5 Hz flicker conditions (either during baseline or 10 Hz tACS). FFT spectra of the baseline data from the inter-trial intervals showed that almost all participants had an individual alpha frequency of 10 Hz or higher. This implies that the P3/P4 lateralization effect may be exclusive to flicker frequencies close to the natural alpha frequency.

An unexpected finding was the different effects of 10 Hz 1 mA tACS across experiments, which consisted of an identical combination of electrical stimulation and visual stimuli. Although the amplitudes of the SSVEPs were enhanced significantly across all electrodes in all experiments the effect on the P3/P4 ratios was different. In experiments one and three, the lateralization effect from the baseline condition was reversed during 10 Hz tACS but the difference between optic-flow and random movement was not significant, whereas in experiment two the lateralization effect was unaffected (i.e. significantly different), as in the baseline condition. There are a number of possible explanations for this finding: Firstly, the two groups in experiments 1 and 2 were 10 different subjects and differences in the response to 10 Hz tACS might have been due to individual subject differences. For example, participants in the first experiment reported a stronger overall sensation of vection (72.5% in experiment 1 and 60.5% in experiment 2) and participants from experiment 1 showed much lower SSVEP amplitudes in the vection baseline condition than participants in experiment 2 (mean POz SSVEP peak-to-peak amplitude in optic flow baseline was 2.7  $\mu$ V in experiment 1 and 4  $\mu$ V in experiment 2). These baseline differences could have led to different effects of the tACS. In addition, as in all tES experiments, many other individual differences such as skull thickness, folding of the cortex, volume of cerebrospinal fluid etc. could have played a part in the variability in the response to brain stimulation across the two groups. Secondly, the experiments were different with regards to the control conditions. The first experiment consisted of two control conditions of tACS also at 1 mA whereas the second experiment contained a control condition at 0.1 mA, which is unlikely to have any effect on neural activity, and a 0.5 mA condition which, as discussed above, may have had different effects to

the 1 mA stimulation. Although we were not looking for off-line effects it is possible that 1 mA stimulation at flanker frequencies, or the 0.5 mA stimulation, had effects on the neural activity that were not obvious from the online measurement of SSVEPs which outlasted the stimulation itself and interacted with the effect in the main condition of interest.

The phase specific analysis did not show any one optimal phase bin across subjects, and the optimal phase bins for each participant were distributed across the bins tested. The latency between visual input arriving at the retina and reaching the visual cortex may not be the same between subjects: additionally the folding of the cortex is unique to each individual and as such different populations of neurons would be preferentially modulated by the current. Furthermore, the baseline SSVEPs did not show any consistent waveform or phase across participants (also most likely due to individual differences in cortical geometry), and as such different effects of any one montage across participants might be expected. This experiment was not designed to investigate phase specific effects in more detail as the 9.9 Hz flicker and 10 Hz tACS drifted in and out of phase at random, future studies will investigate the phase specificity in more systematic manner.

#### *Possibility of Sub-threshold Phosphenes*

An important issue in tACS studies that is controlled for here is the possibility that the electrical stimulation might interact directly with the retina. It has been established that phosphenes during tACS are primarily due to current reaching the retina (Kar & Krekelberg, 2012). Although only two subjects across all three experiments reported intermittently seeing phosphenes as a result of the tACS, there remains the possibility that the current might have had a sub-threshold effect on the retina which biased the cortical response to the flicker. Sub-threshold phosphenes cannot account for the main effect of tACS disrupting the P3/P4 lateralization as they would be no more likely to affect either visual stimulation condition, but sub-threshold phosphenes might have contributed to the overall increase in amplitude of the SSVEPs. There are two reasons why retinal effects are unlikely to have caused the effects we report. Firstly, phosphenes preferentially occur at higher stimulation frequencies, with a maximum phosphene perception in light at 18 Hz (Kanai, Chaieb, Antal, Walsh, & Paulus, 2008), as such we would expect the 12.5 Hz tACS to have been more likely to induce phosphenes, but no significant effects on the SSVEPs were seen in the 12.5 Hz condition. Secondly, the 8.3 Hz and 12.5 Hz tACS in experiment 1 had the same intensity and any one oscillation of the sawtooth wave (sudden switch from anode to cathode) would be equally likely to affect the retina; all frequencies of tACS were drifting in and out of phase with the flickering stimuli and as such all relative phases were included in all stimulation conditions. Therefore, the main effect of tACS when frequencies are closely matched cannot be

explained simply by the stimulation occurring at certain phases but requires the tACS and the flicker to be at an optimal phase for a number of subsequent cycles (see figure 12); as such this effect must be due to an interaction between two oscillators (the cortical response to the flicker and the tACS). Some recent studies have used frontal control sites to exclude the possibility of interaction with the retina; future experiments should employ this approach to fully ensure the effects are not retinal in origin.

### *Limitations*

This study used a comparatively small number of EEG electrodes to be optimally compatible with tACS and this prevents whole-head topography mapping. Future studies will stimulate the left hemisphere as well as right to test whether the effect is hemisphere specific as well as using a frontal control montage to demonstrate specificity of the effect to occipital-parietal areas (see supplementary discussion). Finally, vection sensations were reported using a 4-point scale; finer scales may offer a higher resolution in the future.

### *Conclusion*

Firstly, lateralization of cortical responses to vection induced by optic-flow could be observed in SSVEPs in the alpha range. This is in line with current knowledge on the lateralization of vestibular function in the cortex and the role of alpha oscillations in multi-sensory integration, and is a promising metric for future investigations into visual-vestibular integration.

Secondly, frequency matched tACS was found to be a promising method for manipulating SSVEPs in a cognitive-state specific manner. Frequency and amplitude control conditions demonstrated that the effect of the tACS was only seen when the frequency of stimulation closely matched the flicker frequency and the current intensity was sufficiently high. As this was a large effect, visible at the individual subject level, it is a promising protocol for administering tACS in future studies.



### Example data

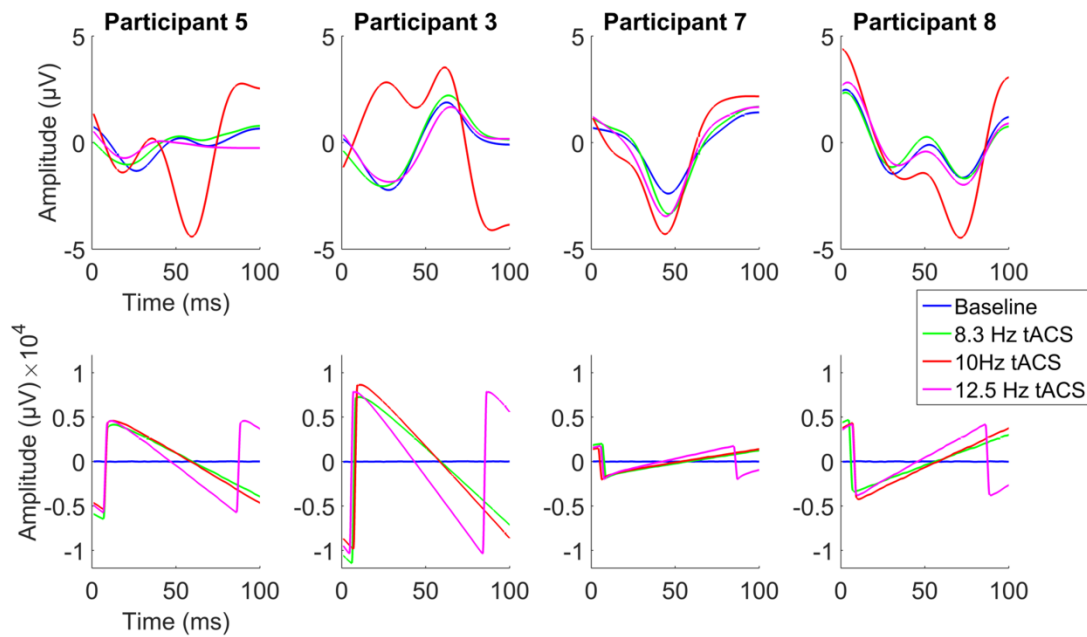
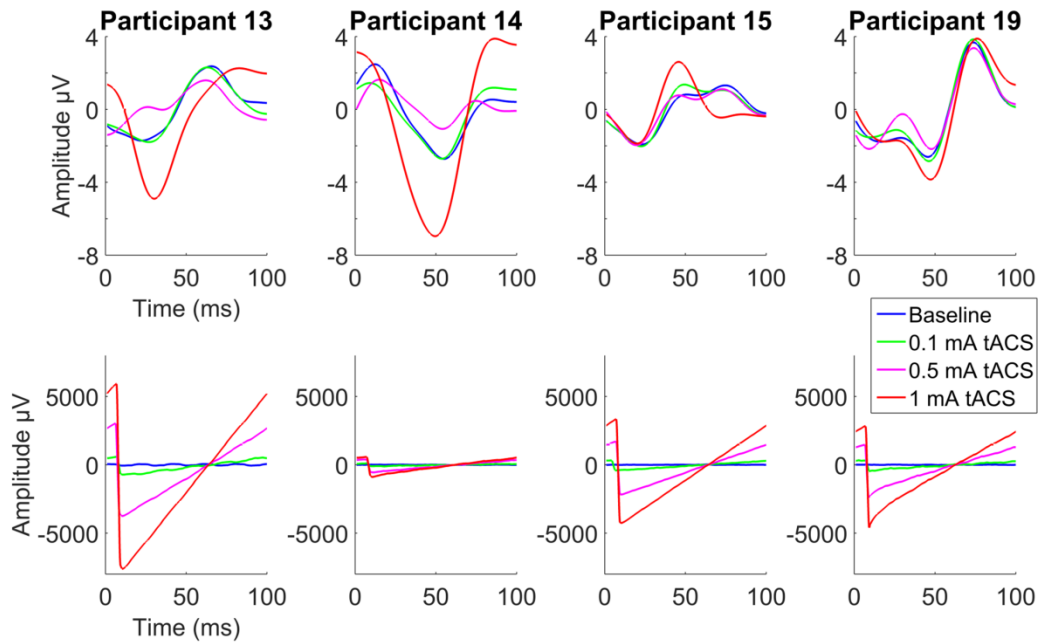


Figure 20: Top row: Example SSVEPs from four participants from experiment 1 from electrode POz, Bottom Row: corresponding example segments of raw data from the same electrode before artefact removal for the same four participants illustrating the variation in size of the artefact across participants. This variation is due to differences in impedance and the resulting voltage of tACS as well as small differences in the relative position of the recording electrode and the electric field of the stimulating electrodes; the closer to the midline of the stimulating electrodes the smaller the artefact, and the polarity of the artefact flips if the recording electrode is either side of the midline (e.g. participants 5 and 7 have opposite polarity). Of note is the overall similarity of SSVEP waveform and amplitudes between baseline and flanker frequency (8.3 Hz and 12.5 Hz) conditions despite the large differences in the size of the artefact across participants. Segments of 10 Hz tACS (red) would be no more likely to leave a residual artefact than the flanker frequencies.



*Figure 21: Top row: Example SSVEPs from four participants from experiment 2 from electrode POz, Bottom Row: corresponding example segments of raw data from the same electrode before artefact removal. The size of the artefact for the weaker tACS is proportionally smaller (0.1 mA, 0.5 mA and 1mA). Of particular note is the similarity between the SSVEPs from the baseline condition and during 0.1 mA tACS (blue and green) despite an artefact hundreds of time larger than the resulting SSVEP being removed; this is good evidence that removing a tACS artefact at 10 Hz does not in itself bias the resulting SSVEPs from 9.9 Hz flicker.*

## **Discussion**

### **5.1. Summary of the two studies**

I will first summarize the two studies which have been presented in this thesis.

The first study (Dowsett & Herrmann, 2016) demonstrated that a sawtooth wave tACS artefact can be removed from EEG recordings and that positive-ramp sawtooth-wave tACS significantly enhanced alpha power during stimulation whereas negative-ramp did not. Three waveforms were tested: sine wave, positive ramp sawtooth and negative ramp sawtooth, each for 10 minutes with peak-to-peak amplitude of 2 mA. The two sawtooth wave conditions were identical in terms of frequency, amplitude and total charge; this demonstrates that current direction and gradient are important factors for the effect of tACS. None of these conditions resulted in a significant increase in alpha power after stimulation, although the positive ramp sawtooth condition after-effect was close to significance, indicating that stimulation for a longer duration might have resulted in a significant increase in alpha power. A sub-group of participants who showed no clear alpha peak with eyes open also showed no peak during tACS, which provides additional evidence that the artefact removal method does not leave a residual peak at stimulation frequency.

The second study used flickering stimuli to induce steady state visually evoked potentials (SSVEPs) at 10 Hz; optic-flow was compared to random dot motion. The first experiment of the second study showed that SSVEPs closely matched in frequency to the frequency of positive-ramp sawtooth-wave tACS were significantly enhanced, whereas tACS at nearby flanker frequencies had no effect. The second experiment replicated the finding of the first (increase in amplitude of SSVEPs closely matched in frequency) and demonstrated that low intensity tACS of 0.1 mA had no effect and intermediate amplitude of 0.5 mA had a variable but non-significant effect on SSVEPs. The third experiment also replicated the finding of the first experiment and demonstrated that tACS at 10 Hz has no effect on SSVEPs at 8.5 Hz. This demonstrates that removing the artefact from 10 Hz tACS can be done successfully.

In the second study we also found a lateralization of SSVEP amplitudes during optic-flow relative to the random dot motion control, which provides additional evidence for a dominant role of the right hemisphere for visual-vestibular integration (in right handers). The lateralisation effect was not present in the 8.5 Hz flicker condition which implies the effect is due to flicker being close to individual alpha frequency. The full power frequency matched tACS (and not the control conditions) also disrupted this lateralization effect in the optic-flow condition, implying the effect is to some extent dependent on the cognitive state.

As the focus of this thesis is on the mechanism of action of tACS and the methodological challenge of combining tACS and EEG, I shall limit this discussion to the potential for tACS to modulate neural oscillations generally and not focus on the specific implications for visual-vestibular processing. The method described here will be useful for the future study of visual-vestibular interactions, but the technique can be also applied to any number of neuro-scientific questions or clinical populations.

## 5.2. Differences between SSVEPs and Endogenous oscillations

An important consideration for the interpretation of the results of the two studies presented in this thesis is the difference in underlying mechanism between resting state endogenous alpha oscillations (first study) and SSVEPs in the alpha range (second study).

The  $p$ -value of the main finding the second study (i.e. the increase in amplitude of SSVEPs,  $p < 0.0001$ ) was much lower than the  $p$ -value of the main finding of the first study (increase in alpha  $p < 0.01$ ), and the effect in the second study is large enough to be visible at the individual subject level. This is broadly in line with the Arnold tongue model which would predict greater enhancement of the oscillation if the frequency of the neural oscillation is closer to the tACS frequency; 10 Hz tACS and 9.9 Hz flicker is very close compared to 10 Hz tACS and the variable individual alpha frequency.

Another factor which might explain the greater effect size in the second study is the fact that endogenous alpha oscillations are considerably larger than SSVEPs. Alagapan and colleagues used both computational models and intracranial recordings in epilepsy patients to demonstrate that smaller oscillations are more susceptible to external alternating currents (Alagapan et al., 2016). Additionally, the after-effect of tACS on alpha power has been shown to only be present when stimulating during eyes-open, when the alpha is lower, and not during eyes-closed, when the alpha oscillation is larger (Neuling et al., 2013).

Another factor to consider in whether or not a neural oscillation is likely to be entrained by tACS, is whether or not the oscillation is generated purely by local cortical-to-cortical connections, or whether there are pacemaker cells in deeper brain regions (e.g. the thalamus) which drive the rhythm of the cortical oscillation (as is the case with sleep spindles). A recent study has argued that the alpha rhythm reflects feedback which propagates from higher to lower cortex and between the cortex and thalamus (Halgren et al., 2017 preprint). It could be the case that endogenous alpha oscillations are partly generated, or sustained, by deeper brain regions where transcranial current is unlikely to have much effect. This might make them less susceptible to changes in amplitude compared to purely

cortical oscillations if connections to deeper brain structures can override an external driving force. Although SSVEPs are driven by external light source, which cannot be influenced by the stimulation and therefore the frequency cannot shift, the amplitude may be more susceptible to tACS because there is no internal pacemaker.

The exact relationship between endogenous neural oscillations and SSVEPs is unclear. One hypothesis is that SSVEPs are the entrainment of neural assemblies already oscillating at the frequency of stimulation (Notbohm et al., 2016); another is that the SSVEP reflects a superposition of the evoked response that would be seen with a single flashed stimuli (Capilla et al., 2011). There is undoubtedly a relationship between the typical frequency bands of naturally occurring oscillations and SSVEPs in those frequency bands as demonstrated by the increased amplitude of SSVEPs (Herrmann, 2001).

An important recent finding, related to this question, is that oscillations directly evoked by TMS show characteristics of endogenous oscillations. Herring and colleagues stimulated the visual cortex with rTMS at alpha frequency and observed the “alpha like” oscillations which are induced (Herring et al., 2015). The authors found that the typical suppression of endogenous alpha, which can be seen when attention is orientated to a particular hemisphere, can also be seen in the TMS evoked “alpha”. This indicates that spontaneous and externally driven oscillations both have a common neural and functional origin.

Further evidence that SSVEPs can have behavioural consequences related to endogenous oscillations at the same frequency comes from a recent study which used a combination of amplitude modulated sound, and luminance modulated movies, to drive the neural response to these stimuli at specific frequencies (Clouter, Shapiro, & Hanslmayr, 2017). Memory for the sound/movie pairs was enhanced when the oscillation luminance/sound was in phase relative to out of phase and baseline. Importantly, this effect was only present at theta frequencies (which are strongly implicated in memory consolidation) and not at slower or faster frequencies. This demonstrates that externally driven neural oscillations can have a complementary function to endogenous neural oscillations.

The primary motivation for the research presented here is to find a reliable bio-marker of the effect of tACS in the EEG, and as such the main findings do not depend on any one theory as to the exact mechanism by which SSVEPs arise. However, the results described here, and the method, could be particularly useful in understanding the exact origins of SSVEPs.

An interesting finding from the second study is that the lateralization effect of optic-flow, relative to random dot motion, did not reach significance for 8.5 Hz flicker. This indicates that the lateralisation effect may be due to an interaction with endogenous oscillations or the network properties of the neurons involved with integrating visual and vestibular cues having

a preference to oscillate in the alpha band, or closer to individual alpha frequency. A setup which can accurately control the frequency of visual flicker, as discussed in the following sections, could be used to test this hypothesis by gradually sweeping the flicker frequency of optic flow and random dot movement and comparing the SSVEPs from different frequencies. Ideally, frequencies both within and beyond the typical alpha range could be tested; if visual-vestibular processing is exclusively related to alpha-band oscillations we would expect to see lateralization only between 8 and 14 Hz.

### 5.3. Artefact removal

Here I will discuss the artefact removal strategy developed in this thesis in the context of methods used by other researchers.

The various strategies to remove the tACS artefact can be divided into two broad types: template subtraction and spatial filters (such as ICA, PCA and beamforming). Spatial filters involve using the data from a large number of electrodes (or sensors in the case of MEG) to estimate the position of a source of noise or artefact, and to remove from all channels the component correlating with the artefact. Spatial filters have been shown to be capable of removing the artefact to such an extent that reconstructed dipolar sources (Soekadar et al., 2013) and changes of brain oscillatory activity such as the increase in alpha amplitude with eyes closed (Neuling et al., 2015) can be recovered even when the stimulation frequency matches the endogenous frequency of interest. However, it has emerged in recent years that the method can leave a residual artefact which can easily be mistaken for neural oscillations (Noury et al., 2016). Typically such studies have only delivered tACS with sinusoidal currents, arguably the most difficult artefact to remove, because a small residual sinusoid in EEG or MEG would be indistinguishable from an on-going neural oscillation. Spatial filters could be combined with non-sinusoidal waves to make identifying a residual artefact easier (as has been done in the two studies presented here, with template subtraction), allowing for rejection of any segments of data where the artefact removal has obviously failed. At the time of writing I am not aware of any attempts to combine spatial filters with non-sinusoidal tACS with steep jumps in current such as sawtooth waves, but this would be a good way to test the effectiveness of any artefact removal method.

Completely removing the artefact is extremely difficult and might be impossible; although it is much easier to detect a residual artefact with square and sawtooth waves, a small artefact below the level of noise could still be present and bias the results of an experiment. The most productive goal of any artefact removal strategy should be to remove as much of the artefact as possible such that any residual artefact is significantly below the level of the neurological

effect of interest. As with TMS studies, the gold standard for any TES experiment should be a control site or control stimulation profile, which has no significant effect on the neuronal response of interest, but which contains a similar artefact. In terms of experimental design this might be the inclusion of a control condition which contains identical tACS but a different cognitive or mental state (e.g. Kasten & Herrmann, 2017). In the first of the two studies presented here we were able to demonstrate that the removal of the artefact does not leave a residual artefact which could be mistaken with neural activity with the negative-ramp sawtooth-wave condition (which did not significantly enhance alpha power) and, more importantly, the group of participants which showed no alpha peak also showed no alpha peak after artefact removal. In the second study we conducted a more extensive series of control conditions to show that different frequencies and low amplitudes at the same frequency have no effect. Observing SSVEPs has a significant advantage in this regard as the number of trials which are averaged (over 500), combined with the fact that the tACS is not exactly phase locked to the flicker, means that any residual artefact, or general noise introduced as a result of stimulating, will be removed in the process of averaging. This resulted in the striking similarities between the baseline SSVEPs and the SSVEPs during stimulation in the control conditions which we were able to observe (see example data, figures 20 and 21). As such, the combination of SSVEPs and tACS is a more convincing artefact removal strategy.

The adaptive template subtraction method used in the studies presented here has a further advantage in that it can be applied to a single EEG electrode, unlike other spatial filter methods such as beamforming which require a full EEG cap or an MEG scanner. This could be important in future development of combined tACS-EEG as there are a number of reasons why fewer electrodes would be desirable. Firstly, the time required to attach 64 EEG electrodes is typically about 30 minutes, this is acceptable for research on healthy participants but could be problematic for clinical studies where patient time is limited, subjects have difficulty sitting still for extended time or if the same subject has to return for regular tACS. Secondly, a long term goal of combined TES-EEG would be that it could be applied in naturalistic conditions with participants/patients moving around, and eventually there may be the possibility of implanted or semi-permanent sub-cutaneous electrodes to deliver individualised stimulation based on EEG at any time, much like a pace-maker for the heart. If this is the case then MEG is not suitable because it is not mobile and a large number of electrodes across the head would not be practical. Lastly, it may be possible to deliver tACS and record EEG from the same electrodes; this could be done with the template subtraction method described here, the only necessary addition would be an EEG amplifier with sufficient range such that the signal would not saturate. This would allow an all-in-one tACS/EEG device that could record from the same location as the stimulation.

Another approach to simultaneous tACS/EEG is amplitude-modulated high-frequency transcranial electrical stimulation (Witkowski et al., 2016). This method involves stimulating with a high frequency sine wave (e.g., 220 Hz) which is modulated by a low frequency carrier wave at the target frequency (e.g. 10 Hz). The goal is that any simultaneous EEG/MEG recordings will only contain an artefact at the higher frequency when viewed in the frequency domain. However, there are some shortcomings: a recent study using a computational model (Negahbani, Kasten, Herrmann, & Fröhlich, 2018) indicates that substantially higher current intensities are needed to produce phase synchronization of on-going oscillations. Furthermore, recent studies have demonstrated that this method can still leave a residual artefact at the amplitude modulated frequency (Kasten, Negahbani, & Herrmann, 2018, Epub ahead of Print) that would not be removed by a simple low-pass filter.

## **Future directions**

The main goal of the research presented in this thesis, and my on-going research, is to improve the method of tACS. Being able to observe neural activity during stimulation is an important first step in optimising the method as it will allow more rapid prototyping of different waveforms, frequencies, intensities and montages. In future research I (and hopefully other researchers) will use the methods developed here to test the relative efficacy of different tACS waveforms, montages, frequencies and intensities. Here I will discuss future directions with a particular emphasis on what online tACS-EEG can tell us about the underlying mechanisms of neural oscillations.

### **5.4. Future directions 1: Frequency**

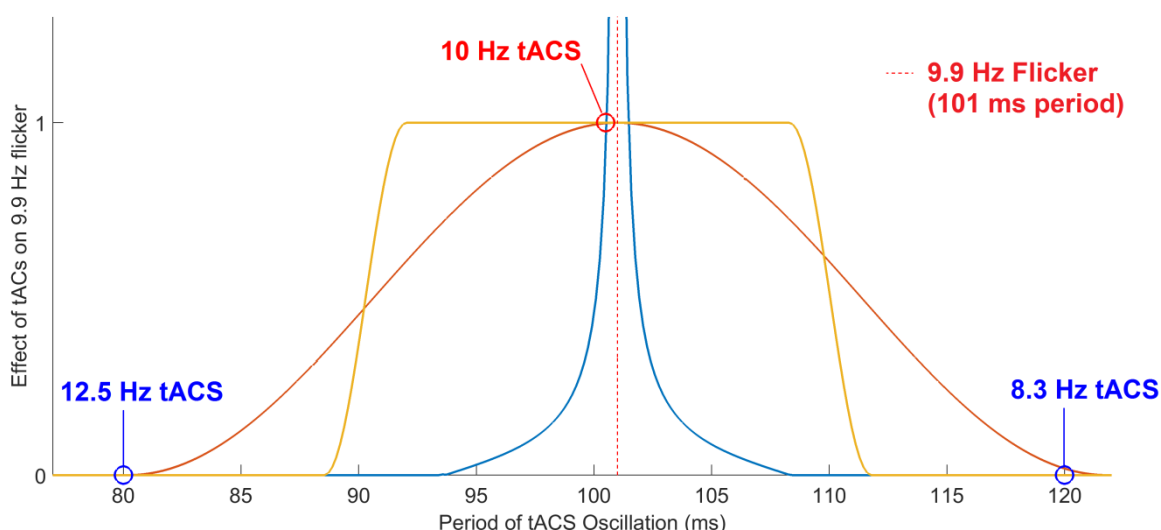
When discussing the underlying mechanisms of various neural oscillators it is important to distinguish between different types of oscillator. One way in which oscillators can be subdivided into types is to observe whether they behave as relaxation oscillators or harmonic oscillators, as features of both can be observed in neural oscillations (Buzsáki, 2006). In Physics, a harmonic oscillator is a system that experiences a restoring force when displaced from equilibrium proportional to the displacement. A feature of harmonic oscillators is that their long-term behaviour can be predicted from the phase. If the speed of an oscillation is not constant within a cycle this is called a non-harmonic oscillator. A common example of a non-harmonic oscillator is a relaxation oscillator which is created by the slow accumulation of some form of energy or potential, and the sudden release after a certain threshold is reached. Single neurons integrate and fire, behaving as relaxation oscillators, whereas larger



populations of neurons can function together in networks to exhibit characteristics of either harmonic or relaxation oscillators. Groups of relaxation oscillators can reset their phases as the result of external perturbations and synchronize, whereas groups of harmonic oscillators poorly synchronize their phases (Buzsáki & Draguhn, 2004).

Manipulating neural oscillations with external stimulation and observing the reaction is a promising approach to understanding the neuronal circuits that produce the rhythmic activity. Here I will discuss how the methods presented in these experiments, in particular the second study, can be used to this end in future research.

The first experiment, in the second study, clearly shows a significant enhancement of SSVEPs, only when the flicker and tACS frequencies are closely matched (10 Hz tACS has an effect on 9.9 Hz flicker, 8.3 Hz and 12.5 Hz tACS does not). A question which is raised by this finding is: how close in frequency does the tACS need to be to amplify the SSVEP, and how does the effect vary as the flicker frequency approaches the tACS frequency? Future experiments addressing this question could provide interesting evidence as to the underlying mechanism of SSVEPs. There are two ways this could be achieved: firstly by keeping the tACS frequency constant and utilising a setup which can accurately control the frequency of visual flicker such that it can be set to any arbitrarily close frequency to the tACS; possibilities include LED lighting, LCD shutter glasses or a rotating mechanical shutter to rhythmically block a light source. A second possibility would be to keep the flicker frequency constant and to move the tACS frequency progressively closer. There are several possible outcomes (illustrated in figure 22).



*Figure 22: Illustration of the results of experiment 1 in the second study (tACS frequency control) and three hypotheses as to how intermediate tACS frequencies might affect the 9.9 Hz SSVEP (which has a period of approximately 101 ms, red dashed line).*

Results so far show a significant effect of 10 Hz tACS (period of 100 ms, red circle, figure 22) and no effect, relative to baseline, for 12.5 Hz (period 80 ms) and 8.3 Hz (period 120 ms, blue circles, figure 22). Future experiments will test a range of tACS frequencies between 8 and 12 Hz, the results will provide an interesting insight as to how tACS and SSVEPs interact. Shown are three possible outcomes.

Blue: the effect of the tACS increases exponentially as the frequency approaches the flicker frequency, resulting in the effect only being present in a narrow band around the flicker frequency. In this scenario there is a maximum effect, greater than the effect found in the data so far, when the tACS and flicker are matched exactly. This is reminiscent of an effect seen in physics, specifically the response amplitude as the frequency of a driven damped simple harmonic oscillator approaches its resonant frequency.

Red: the effect of tACS increases as the frequency approaches the flicker frequency with the effect size following a bell shaped curve, peaking at the flicker frequency. This scenario would be consistent with the SSVEP being the result of a large number of neurons with a range of preferred frequencies around the driving (flicker) frequency; as the tACS approaches the flicker frequency an increasingly larger number of neurons can be recruited into the oscillation.

Yellow: the effect of the tACS may form a plateau in a range around the flicker frequency within which the tACS has the same effect. If this is the case, one interpretation as to the effect of the tACS would be that it is due to the sudden jump of the sawtooth wave happening anytime within a certain phase of the SSVEP, and there is no additional benefit with regards to where in this time window. This might be the case if the SSVEP contains an “up phase” during which more neurons are firing and any external electrical field during this time window would add to the amplitude of the oscillation up to a certain maximum. This outcome would also be consistent with the Arnold Tongue model, with a sharp boundary between entrainment and no entrainment with a fixed stimulation intensity.

Another way to think of the relationship between the SSVEPs and the tACS is the number of successive cycles for which the two oscillators are in a particular relative phase window. This number increases exponentially as the frequency of stimulation approaches the flicker frequency, similar to the blue line in figure 22.

These three possibilities are just broad examples which are simple to model; there could be any number of possible outcomes which are combinations of the three shown here, or exhibit non-symmetrical response curves (e.g. the effect of the tACS being slightly above the flicker frequency might be different to the effect if it is slightly below the flicker frequency, indicating something similar to a relaxation oscillator). The main point here is to highlight the potential

for future experiments using this paradigm to provide insights as to the neuronal mechanism behind SSVEPs.

This experimental paradigm could also be extended to investigate endogenous neural oscillations, although this is technically more difficult as, unlike SSVEPs, naturally occurring neural rhythms are not a fixed phase or frequency and these parameters change over time. If real-time artefact removal and closed loop tACS becomes technically feasible in the future this might be possible.

## **5.5. Future directions 2: Waveform**

When observing neural oscillations in EEG/MEG it is important to be aware of the limitations of commonly used analysis methods. A neural oscillation can often appear as an approximately sinusoidal curve when it is actually non-sinusoidal, due to band-pass filters which gives the signal a more smoothed shape. Although some oscillations, most notably the posterior alpha rhythm, are approximately sinusoidal, many neural oscillations are not and this can cause spurious results with traditional analysis methods (Jones, 2016). Recently it has been suggested that investigating the non-sinusoidal properties of neural oscillations can provide a valuable insight as to the assemblies of neurons which constitute them (Cole & Voytek, 2017). The studies presented here, and subsequent future research, might compliment this line of reasoning by observing the effect of different stimulation waveforms on neural oscillations. Here I will discuss some possibilities for future research and caveats.

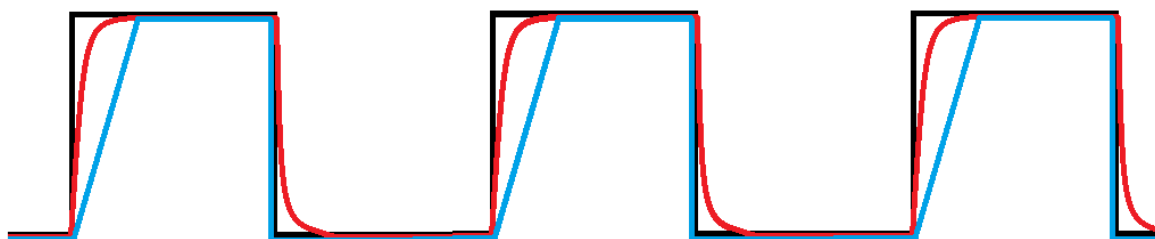
Studies with intracranial recordings in awake ferrets have shown that ramps of increasing voltage with a steeper gradient resulted in increased neural firing, relative to ramps with a low gradient but which reached the same maximum voltage (Fröhlich and McCormick, 2010, Supplementary Material). This demonstrates that it is not only the total amount of current but also the rate of change of current which modulates neural firing. This led to the current hypothesis that tACS waveforms with steeper gradients would be better suited to entraining neuronal oscillations in humans. To test this we first stimulated with the steepest gradient possible: an instantaneous rise or sudden jump in current. In the two studies presented in this thesis sawtooth waves were chosen for two reasons: firstly to simplify the problem of artefact removal, and secondly to limit the sudden jump in current to one direction. Many other waveforms could be used for TES, and these possibilities should be explored thoroughly in future research. An obvious starting point would be square waves, but an additional interesting variation would be to use trapezoid shaped waveforms to vary the steepness of the rising or falling current. Square waves contain sudden jumps in current in both directions, unlike sawtooth waves which contain a sudden jump in one direction and a

gradual change in the other. This is similar to the difference between mono-phasic and bi-phasic pulses in TMS. Most TMS devices use a large current which flows around the coil in one direction, producing a magnetic field with one orientation and a resulting electrical field in one direction, which is immediately followed by a current in the other direction resulting in an induced current in the opposite direction. Some TMS devices allow for only one of these currents to reach the coil and therefore only produce current in one direction; this “mono-phasic” TMS results in direction-specific effects (Jung et al., 2012). Different current directions are thought to activate different populations of neurons (Kammer et al., 2001). The most common usage of TMS is to create a so-called “virtual lesion” by disrupting the normal functioning of an area of cortex by causing neurons to fire indiscriminately and adding noise to the system; for this purpose a biphasic pulse may be more suitable as, in theory, twice as many neurons are affected. This might also be true for square wave tACS, although the mechanism of action is different; tACS modulates the probability of neurons firing rather than directly inducing action potentials.

On the other hand it may be desirable to limit the sudden jump in current to one direction. Varying the current directions in tACS, by changing the position of the return electrode from left shoulder to right shoulder, has been found to significantly change the effect of tACS on tremor (Mehta, Pogosyan, Brown, & Brittain, 2015). In tDCS, changes in motor excitability have been reported to be specific to orientation of current flow (Rawji et al., 2018). It may be useful to optimize tACS for each individual by keeping the electrode positions constant and varying the waveform such that sudden jumps in current occur in different directions; this could be done with positive and negative ramp sawtooth, but other hybrid waveforms could be used.

An important factor to consider here is the effect of capacitance. When any electrical current passes from one medium to another with different conductance there will be some charge stored. For example, the skin acts like a capacitor in that it allows more current to flow if a voltage is changing rapidly (Fish & Geddes, 2009). Conductivity ratio's for scalp, skull and brain are commonly modelled as 1, 1/80, 1 respectively (i.e. skull is a far worse conductor). For these reasons it is important to consider that the rate of change in stimulating current at the scalp is unlikely to reflect the rate of change in the current that reaches the cortex in the case of sudden changes in current (such as sawtooth waves and square waves) because the skull will act as a low-pass filter. An instantaneous change in current at the scalp will reflect the maximum rate of change possible with TES, and this will be lower than the rate of change of current induced by TMS and intracranial stimulation because current does not pass through the skin or skull.

This change in current gradient could be estimated using computational models, but would ideally be tested in the awake human brain as individuals will vary in their exact conductivity values. A possible experimental design would be to test tACS waveforms with a range of rising or falling gradients including a sudden rise (or fall) in current, and a similar waveform with a less steep rising edge. If these two conditions result in a similar cortical response then a valid conclusion would be that the skin/skull is acting as a low-pass filter; testing a series of different gradients could in theory provide an estimate of the filter properties acting on current reaching the cortex (figure 23).



*Figure 23: An illustration of square wave stimulation (black) and a typical output that might be observed after a low-pass filter (red). This is a current profile that might be observed at the cortex if the skin and skull act as a low-pass filter. If this is the case then an alternative waveform without a steep rising edge (blue) would be less affected by the low-pass filter properties.*

By varying the steepness of this rising edge, and the observing the resulting effect on frequency matched SSVEPs, it may be possible to observe the maximum possible rate at which transcranial current can be delivered to the cortex.

A further property of square-wave stimulation that can be manipulated independently of the frequency is the duty cycle; the ratio of time the current is ON vs. OFF. For example a duty cycle of 0.5 would reflect an equal amount of time that the current is ON and OFF (or positive and negative if the current is alternating). For a 10 Hz square wave tDCS this would be 50 ms ON and 50 ms OFF. A duty cycle of 0.1 would be a shorter pulse of current, for a 10 Hz wave this would be 10 ms ON and 90 ms OFF etc. An interesting line of research would be to investigate the effect of TES with different duty-cycles, as the frequency and peak amplitude could be kept constant, but the total amount of current delivered would be varied. This could provide insight into how such waveforms are enhancing neural oscillations; if only a short burst is necessary to observe the effect seen in the current two studies, we could conclude that the effect is the result of a sudden change in current at the critical point in the on-going neural oscillation. On the other hand, if shorter pulses of current have progressively less effect then we could conclude that the current needs to be high for a sufficient amount of time.

### 5.6. Future directions 3: Current Intensity

The artefact removal method could also be used in future studies to individualise the stimulation intensity by gradually ramping up the current. It could be the case that exactly matching the frequency of SSEPs and tACS requires significantly lower current intensities to achieve an effect on neuronal firing than are traditionally used in TES studies. Indeed, the Arnold tongue model would predict exactly this outcome; as the driving frequency approaches the frequency of the endogenous oscillation the force required for entrainment/enhancement decreases. However, there must be some lower threshold, below which there is no effect. This could be observed by exactly matching the frequency of the tACS and flicker and lowering the current until no effect is observed. This threshold may, or may not, be lower than the 0.1 mA condition, which had no effect in experiment 2 of the second study, as the flicker was not exactly 10 Hz.

It is possible that there is a non-linear relationship between current intensity of TES and the effect on neuronal activity. This could be due to a different sensitivity of excitatory and inhibitory synapses to different intensities of stimulation (Moliadze et al., 2012). Future experiments could test a range of intensities on frequency matched SSVEPs to test for any non-linear relationship.

### 5.7. Future directions 4: Phase

An interesting finding from the second study was the phase specificity of the tACS on the SSVEP amplitude. The 10 Hz tACS condition in the first experiment, the 1mA (10 Hz) condition in the second experiment and the 10 Hz tACS/9.9 Hz flicker condition in the third experiment all showed significant increases in the amplitude of the evoked potentials at certain phases. This strongly implies that the main effect, i.e. the increase in amplitude, was driven by time periods when the tACS and flicker were in particular relative phase. These experiments were not designed to investigate phase specific effects as the 9.9 Hz flicker and 10 Hz tACS drifted in and out of phase at random. The optimal phase bin was different across participants. Furthermore, for some participants there were large increases in amplitude from non-adjacent phase bins with relatively small effect in the intermediate phase bins; perhaps indicating the SSVEP is composed of separate populations of neurons, each with a different optimal phase relationship. This could be due to either different latency between the signal from the retina arriving at different patches of cortex, or alternatively to different patches of cortex having different orientations relative to the stimulating electric field. Future experimental setups should aim to control the phase of frequency-matched tACS and flicker precisely, to properly test this hypothesis.

## 5.8. Limitations

One limitation of the studies presented here is the relatively small number of EEG electrodes. This was mainly due to the tACS artefact being so large that the signal would “clip” (i.e. reach the limit of the amplifier and produce a flat line) for any EEG electrodes close to the stimulating electrodes. During TES/EEG, the smallest artefact is at the mid-line between the stimulating electrodes and this was the optimal position chosen to avoid clipping. This problem can be reduced to some extent with EEG amplifiers with a larger range; the EEG amplifiers used for the two studies presented here were 16-bit amplifiers, many modern EEG amplifiers are 24-bit and as such have a much larger range of values for the same resolution. Future studies using combined tACS/EEG should utilize such equipment to maximise the number of electrode positions which can be recorded from. In an ideal case a full cap of 64 electrodes would be usable such that source localisation could be used, however such a large number of electrodes may cause a different current flow of the TES, as the highly conductive gel provides additional paths the current can take across the scalp.

Another limitation of the current research is there is a lack of computational/animal modelling of the effects of tACS with waveforms other than sine waves. A number of groups have modelled the effect of sinusoidal tACS with neural networks (e.g. Ali, Sellers, & Frohlich, 2013; Alagapan et al., 2016). Such models can provide useful insights into why tACS is, or is not, able to entrain neural activity depending on different parameters of the stimulation. Future research could reproduce these models with the simple variation of changing the sinusoidal stimulation to sawtooth-waves, square waves or any of the variations discussed here.

Research using animal models has a significant advantage over research on healthy human participants in that inter-cranial and single unit recordings *in vivo* are possible. Any of the experiments described here could be performed on rodents. This could potentially reveal a lot about the underlying mechanism of the effects seen here that could not be deduced from transcranial human studies.

## 5.9. General conclusions and outlook

The overall conclusion of the two studies presented here is that sawtooth-wave tACS can be combined with EEG, the artefact can be successfully removed, and positive ramp sawtooth tACS has a significant effect on both alpha oscillations and frequency matched SSVEPs in the alpha range.

Specifically, in terms of the research questions presented in the introduction:

- tACS can modulate neural oscillations.
- The artefact can be removed with no evidence of a residual artefact in the control conditions.
- We have a reliable biomarker of tACS to help optimize and guide the development of the method; SSVEPs and tACS are particularly promising in this regard.
- Positive ramp sawtooth waves are more optimal than negative ramp sawtooth waves for the montage used here.
- SSVEPs need to be within approximately 1.5 Hz of the tACS frequency for there to be an effect, with the parameters used here.
- A current intensity of more than 0.1 mA is needed to manipulate frequency matched SSVEPs, with the parameters used here.

The second study is part of an on-going research project using tACS/EEG to investigate the role of neural oscillations in visual-vestibular interactions. An eventual goal is to transfer this methodology to a fully mobile setup such that SSVEPs can be recorded while participants/patients walk around; SSVEPs may be particularly useful in this regard because the excellent signal-to-noise ratio significantly reduces the problem of movement artefacts.

This method will be useful for selectively enhancing SSVEPs at certain frequencies, which would have many applications, for example, in brain computer interfaces. More generally, this method will be a valuable tool for investigating the underlying mechanisms of neural oscillations and to help push the boundaries of how we can manipulate them and how we can help correct abnormal brain activity and enhance cognitive function.



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<http://doi.org/10.1371/journal.pone.0013766>

## Statement of contributions

I hereby confirm that James Dowsett contributed to the aforementioned studies as stated below:

### First Study

“Transcranial Alternating Current Stimulation with Sawtooth Waves: Simultaneous Stimulation and EEG Recording.”

Author Contributions:

James Dowsett formulated the research question, participated in the study design, carried out the experiments, analysed the data and wrote the final paper.

Christoph Herrmann formulated the research question, guided the study design and data analysis and edited the final paper.

### Second Study

“Lateralized EEG responses during visually induced vection from visual flicker in the alpha range and subsequent modulation using frequency matched tACS.”

Author Contributions:

James Dowsett formulated the research question, designed the study, carried out the experiments, analysed the data and wrote the final paper.

Christoph Herrmann, Marianne Dieterich and Paul Taylor guided the study design and data analysis and edited the final paper.

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Signature of Supervisor

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Date

## Statement of good scientific practice

I (James Dowsett) hereby confirm that the work presented in this thesis was completed independently by myself, using only the resources presented in this document.

This thesis was conducted according to the Carl von Ossietzky Universität guidelines for Good Scientific Practice (Leitlinien guter wissenschaftlicher Praxis).

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Signature

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Date

## Curriculum vitae - James Dowsett

### Date and place of birth

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### Previous employment

2011 – 2012: Research assistant, Durham University Cognitive Neuroscience Research Unit

2004 – 2011: Musician

### Peer reviewed journal publications

Kasten FH, Dowsett J and Herrmann CS (2016) **Sustained Aftereffect of  $\alpha$ -tACS Lasts Up to 70 min after Stimulation**. *Frontiers in Human Neuroscience*, 10:245. doi: 10.3389/fnhum.2016.00245

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**Revealed Using Functional Magnetic Imaging and Transcranial Direct Current Stimulation.** PLoS ONE, 9(4): e93767. doi:10.1371/journal.pone.0093767

#### **Published conference abstracts**

**Cognition and higher vestibular disorders: developing tools for assessing vection** (2017) Dowsett, J., McAssey, M., Dieterich, M. et al. J Neurol, 264(Suppl 1): 45

**Transcranial alternating current stimulation with sawtooth waves: a novel method for simultaneous stimulation and EEG** (2015) Dowsett, et al. Brain Stimulation, Volume 8, Issue 2, 352 - 353

**An investigation into the effects of transcranial alternating current on alpha oscillations using simultaneous stimulation and EEG** (2014) Dowsett, et al. Brain Stimulation, Volume 7, Issue 2, e13

#### **Conferences, Posters and Talks**

2018 – Perturbing and Enhancing Perception and Action using Oscillatory Neural Stimulation, Cambridge, UK

2017 - Brainbox Brain Stimulation Conference, London, UK

2017 – 2nd International Brain Stimulation Conference, Barcelona, Spain

2016 - 6th International Conference on Transcranial Brain Stimulation, Göttingen, Germany

2015 - 1st International Brain stimulation Conference (Invited for oral presentation), Singapore

2014, 2013, 2012, 2011 - Magstim Neuroscience Conference (2013 poster prize winner) Oxford, UK

2013 - NYC Neuromodulation Conference, New York

2013 - 5th International Conference on Non-invasive Brain Stimulation, Leipzig, Germany

2012 - 16th World Congress of Psychophysiology, Pisa, Italy

2012 - British Association for Cognitive Neuroscience Conference Newcastle, UK

#### **Invited reviewer for journals**

Frontiers in Human Neuroscience

Brain Stimulation