Modulation of Neural Oscillations and Associated Behaviour by Transcranial Alternating Current Stimulation (tACS)

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A thesis submitted in fulfilment of the requirements for the Degree of

Doctor of Philosophy

School of Psychology

College of Science and Engineering

University of Glasgow

February 2017

Abstract

Transcranial alternating current stimulation (tACS) is a non-invasive brain stimulation method that involves the application of weak electric currents to the scalp. tACS has the potential to be an inexpensive, easily administrable, and well-tolerated multi-purpose tool for cognitive and clinical neuroscience as it could be applied to establish the functional role of rhythmic brain activity, and to treat neural disorders, in particular those where these rhythms have gone awry. However, the mechanisms by which tACS produces both "online" and "offline" effects (that is, those that manifest during stimulation and those that last beyond stimulation offset) are to date still poorly understood. If the potential of tACS is to be harnessed effectively to alter brain activity in a controlled manner, it is fundamental to have a good understanding of how tACS interacts with neuronal dynamics, and of the conditions that promote its effect. This thesis describes three experiments that were conducted to elucidate the mechanisms by which tACS interacts with underlying neural network activity.

Experiments 1 and 2 investigated the mechanism by which tACS at alpha frequencies (8 - 12 Hz, α-tACS) over occipital cortex induces the lasting aftereffects on posterior α-power that were previously described in the literature. Two mechanisms have been suggested to underlie alpha power enhancement after α-tACS: entrainment of endogenous brain oscillations and/or changes in oscillatory neural networks through spike timing-dependent plasticity (STDP). In Experiment 1, we tested to what extent plasticity can account for tACS-aftereffects when controlling for entrainment characteristics. To this end, we used a novel, intermittent α-tACS protocol and investigated the strength of the aftereffect as a function of phase continuity between successive tACS episodes, as well as the match between stimulation frequency and individual alpha frequency (IAF). Alpha aftereffects were successfully replicated with enhanced α-power after intermittent stimulation compared to sham. These aftereffects did not exhibit any of the expected characteristics of prolonged entrainment in that they were independent of tACS phase-continuity and did not show stable phase alignment or synchronisation to the stimulation frequency. These results indicate that prolonged entrainment is insufficient to explain the

aftereffects and suggest that the latter emerge through some form of network plasticity.

To clarify the nature of these plasticity mechanisms, we then aimed to assess whether STDP could explain the α-power increase. We developed a conceptual STDP model that predicted bi-directional changes in α-power depending on the relative mismatch between the tACS frequency and IAF. After observing in Experiment 1 that tACS at frequencies slightly lower than the IAF produced α-enhancement, Experiment 2 used a similar intermittent protocol that manipulated tACS frequency to be either slightly lower or higher than IAF to respectively enhance or suppress α-activity. In addition, a control condition with continuous stimulation aimed to replicate previous results from other groups. However, we did not observe a systematic α-power change in any of the active conditions. The lack of consistency between the two experiments raises concerns regarding the reproducibility and effect size of tACS aftereffects.

The third experiment investigated the mechanism of online effects and tested predictions that were based on the assumption that entrainment is the underlying process mediating behavioural changes during tACS. We capitalised on two well-described phenomena: firstly, the association between α-power lateralisation and visuospatial attention, and secondly, the fluctuation of perceptual performance with α-phase. Specifically, the experiment tested whether event-related α-tACS applied over right parieto-occipital cortex can induce a visuospatial bias in a peripheral dot detection task that would reflect α-power lateralisation, and whether detection performance depends on the phase of the tACS waveform. In control trials either no tACS or 40 Hz-tACS (gamma) was applied to make use of the putative opposing roles of alpha and gamma oscillations in visual processing. As expected from lateralised enhancement of alpha oscillations, visual detection accuracy was weakly impaired for targets presented in the left visual field, contralateral to tACS. However, this effect was neither frequency-specific nor waveform phase-dependent. Therefore, it is unlikely that the negative effect of tACS on visuospatial performance reflects entrainment.

Overall, the results of these experiments only partially met our hypotheses. Experiment 1 produced the α-enhancement that was expected

based on the literature while the follow-up experiment failed to reproduce these results under similar conditions. This outcome demonstrates at best that tACS aftereffects on α-activity are not robust, may vary widely across individuals, and might be extremely sensitive to small changes in experimental parameters and state variables. The results of the third experiment call into question the assumption of online entrainment as basis for the observed behavioural effect. These findings point to the need for improved methodology, for more systematic and exhaustive exploration of the relative effects of tACS across different parameter settings, tasks, and individuals; and for the replication of promising but thus far often anecdotal results. They also inspire guidelines for more informative experimental designs.

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Acknowledgement

This work was supported by a scholarship from the College of Science and Engineering, and a Wellcome Trust Award to Gregor Thut and Joachim Gross [grant number 098434, 098433].

Thank you…

…Gregor, for your enthusiasm, for not doubting me for one second, for all those heated discussions and for challenging my beliefs. I think our weirdly bipolar dynamics have made this thesis as good as it can be;

…Joachim, for valuable analytical advice and invaluable moral support;

…Flavio Fröhlich and his team, for providing an opportunity to escape and for reminding me that science is awesome;

…Tibor Nánási & Amanda Ayoub for assistance in data collection;

…Gavin Paterson, John Shaw, Nosrat Mirzai, for taking the time to help with all those technical problems. I know it's your job but the learning curve was steep and I'm grateful for every bit of wisdom.

…Steph, Gemma, Yulia and the rest of the lot, for assisting with experiments, for coffees & pints, bad movies, gigs, walks; in short, for creating opportunities to have a LIFE. Oh, and the occasional proof reading.

…Kevin, Sabina, Kasia, Jessica, Fei: without your distraction I would have been finished at least half a year earlier but it wouldn't have been half as much fun.

…Bruno, for that pep talk and hug when quitting seemed so much easier than carrying on;

…Lachlan, for feeding me, for being my guinea pig, and for picking me up again and again;

…Gavin, for simply being &%$£*#@ wonderful;

…Scottish hills; because whether I was enjoying a wee scramble, or strolling around with the sun on my face, or being swept away by gales and torrential rain, you always cleared my head and kept me from going insane.

…Papa, für unermüdliche Unterstützung und IT support.

…und Mama; für deine bedingungslose Hilfe dabei, das zu werden, was ich sein wollte. Das hier ist für dich, denn ohne dich wär ich nicht hier. Ich hoffe du bist stolz auf mich. Du fehlst mir. Alles Gute zum Geburtstag.

Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Alexandra Vossen

Abbreviations

EEG	electroencephalography
MEG	magnetoencephalography
tES	transcranial electrical stimulation
tACS	transcranial alternating current stimulation
(o)tDCS	(oscillatory) transcranial direct current stimulation
tSOS	transcranial slow oscillation stimulation
tRNS	transcranial random noise stimulation
(r)TMS	(repetitive or rhythmic) transcranial magnetic stimulation
iTBS	intermittent theta-burst stimulation
NIBS	non-invasive brain stimulation
IAF	individual alpha frequency
ISF	individual stimulation frequency
VAS	visual analogue scale
LTP/LTD	long term potentiation/depression
STDP	spike timing-dependent plasticity
VF	visual field

List of Publications

The results in Chapter 2 have been published as:

Vossen, A., Gross, J., & Thut, G. (2015). Alpha power increase after transcranial alternating current stimulation at alpha frequency (α-tACS) reflects plastic changes rather than entrainment. *Brain Stimulation, 8*(3), 499–508. http://dx.doi.org/10.1016/j.brs.2014.12.004

These data were also presented orally at *Magstim Neuroscience Conference*, Oxford, May 2015.

The results in Chapter 3 were presented orally at *Psychologie und Gehirn*, Frankfurt, June 2015.

The results in Chapter 4 were presented orally at *Entrainment of Brain Oscillations Conference*, Delmenhorst, September 2015.

Material researched for this thesis has also been published as:

*Veniero, D., *Vossen, A., Gross, J., & Thut, G. (2015). Lasting EEG/MEG aftereffects of rhythmic transcranial brain stimulation: Level of control over oscillatory network activity. *Frontiers in Cellular Neuroscience, 9*, 477. http://doi.org/10.3389/fncel.2015.00477 (*equal contribution)

Chapter 1. General introduction

Non-invasive electrical brain stimulation as a form of therapy appears to have been employed in human patients at least as early as during the Roman empire, from whence stems the first recorded use of electric fish as remedy against chronic headaches (Kellaway, 1946). Since the Romans we have come a long way, and such "electro-ichthyic" stimulation has been gradually replaced by technologically more sophisticated methods aimed at increasingly more subtle targets. Modern approaches involving electric currents intended to alter brain activity are collectively known as transcranial electrical stimulation (tES) which include transcranial direct current stimulation (tDCS), transcranial random noise stimulation (tRNS) and transcranial alternating current stimulation (tACS).

During the past decade there has been a massive upsurge in the interest in neuromodulation with tES and, as a consequence, in the biological basis of its effects on brain activity and behaviour. Many studies have been published that report concurrent or outlasting changes in neuro-electrical activity (see section *Evidence for tACS-induced entrainment of neural activity*, this chapter), cerebral blood flow (Stagg et al., 2013), neurotransmitter concentration (Stagg et al., 2009), and most prominently perception, cognition and memory (see section *Behavioural effects of tACS*, this chapter). Thus, there seems to be little doubt that tES is effective in inducing physiological changes that are translated into changes in behaviour, and accordingly the optimism is high for the deployment of tES in a number of experimental and clinical applications. These include the development of effective therapeutic tES interventions for a wide range of pathologies, for causal manipulation of brain activity in order to explain the link between neuronal action and specific aspects of human behaviour, and for the improvement of skills and well-being through neuro-enhancement.

There are many possible targets for tES. Some of these targets are behaviourally defined, such as working memory capacity or abstract reasoning. Alternatively, the target can be neurally defined, for instance in terms of cortical excitability or, as will be the topic of this work, neural oscillations, which reflect synchronous activity across populations or networks of neurons. This thesis investigates specifically how transcranial alternating current

stimulation (tACS) at physiologically relevant frequencies interacts with such oscillations.

The concept of neural oscillations is usually credited to Hans Berger, who was the first physiologist to describe the rhythmic waves in the human encephalogram as "alpha" and "beta" waves (Berger, 1935). Somewhat ignored as neural background noise during the high tide of the event-related potential (ERP) paradigm, neural oscillations have meanwhile been found to be modulated in a task- and state dependent fashion in a large number of cognitive, perceptual, and motor processes, and to correlate with a variety of behavioural measures. Oscillations have therefore now been accepted to be an integral part of neural information processing (Buzsáki, 2006; Thut, Miniussi, & Gross, 2012). In accordance with the putative importance of neural rhythms for normal brain functioning, abnormal changes in oscillatory activity have been associated with several neuropathologies, including Alzheimer's disease, Parkinson's disease, autism spectrum disorder, and schizophrenia (Schnitzler & Gross, 2005; Uhlhaas & Singer, 2006).

Knowledge of the relationship between brain rhythms and behaviour is to date predominantly derived from electroencephalography (EEG) and magnetoencephalography (MEG), which respectively record electrical and magnetic brain activity on the scalp surface. However, this type of research is intrinsically correlational. Causal intervention is required to show that these oscillations are not only epiphenomenally related to behaviour but also functionally relevant. If such a functional role for oscillations exists, manipulating aberrant synchronicity towards normal levels could in theory alleviate symptoms or even reinstate the normal healthy state. Therefore, oscillatory activity is an attractive target for a variety of applications both in the study of cognition and for the treatment of neural disorders, and by extension a popular target for tES. However, much like the early Greeks and Romans were puzzling over the mechanism and effects of electric shocks induced by their gill-bearing narcotics, so are the mechanisms and effects of tES and their interaction with neural tissues in healthy, functional networks under varying brain states still poorly understood. Naturally, it is important to understand the impact of

electrical stimulation on brain activity, in order to design rational interventions and minimize potential risks.

The aim of this thesis is to increase our understanding of the mechanisms leading to both "online" and "offline" neural and behavioural effects. Online effects are defined as the direct, immediate and transient effects of tACS during active stimulation, which should be especially useful in the causal study of cognition and perception. Offline effects refer to those changes that last beyond stimulation and warrant hope that tACS could provide a versatile therapeutic tool. Chapter 1 sets the stage by giving an overview of the most important concepts in this work. It introduces the alpha rhythm, which constitutes the primary neural target for tACS in the experiments described in this thesis. It also introduces tACS, including a description of the basic technical aspects and a review of our current understanding of the mechanism by which it induces neural effects, namely entrainment of neural oscillations. Following a thorough discussion of the background, Chapters 2 - 4 then present three experiments that were designed to test some of the assumptions that are frequently evoked to explain how tACS exerts its effects. Chapter 5 attempts with a general discussion to link the findings from these experiments and to integrate them into the bigger picture of and beyond the existing literature.

The alpha rhythm

To recap briefly, the spectrum of neural oscillatory activity as measured by EEG or MEG is often subdivided into different frequency bands with more or less arbitrary and somewhat variable boundaries (Başar, Başar-Eroglu, Karakaş, & Schürmann, 2000; Ernst Niedermeyer, 1999; Rosanova et al., 2009; Schürmann & Başar, 2001; Wang, 2010). These frequency bands are (arbitrarily) labelled with Greek letters and include: Delta (δ, roughly up to 4 Hz), theta (θ, 4 - 8 Hz), alpha (α, 8 - 12 Hz), beta (ß, 13 - 30 Hz), and gamma (γ, greater than 30 Hz). Sometimes the high frequency (ripple) range above 100 Hz is included. These frequency bands may be subdivided (e.g., "slow delta" of oscillations of less than 1 Hz, low and high alpha, beta and gamma sub-bands) depending on their functional relevance. This work is primarily concerned with activity in the α-band, specifically those rhythms that are distinctly visible in the time series recorded by sensors located over the posterior scalp (Figure 1.1).

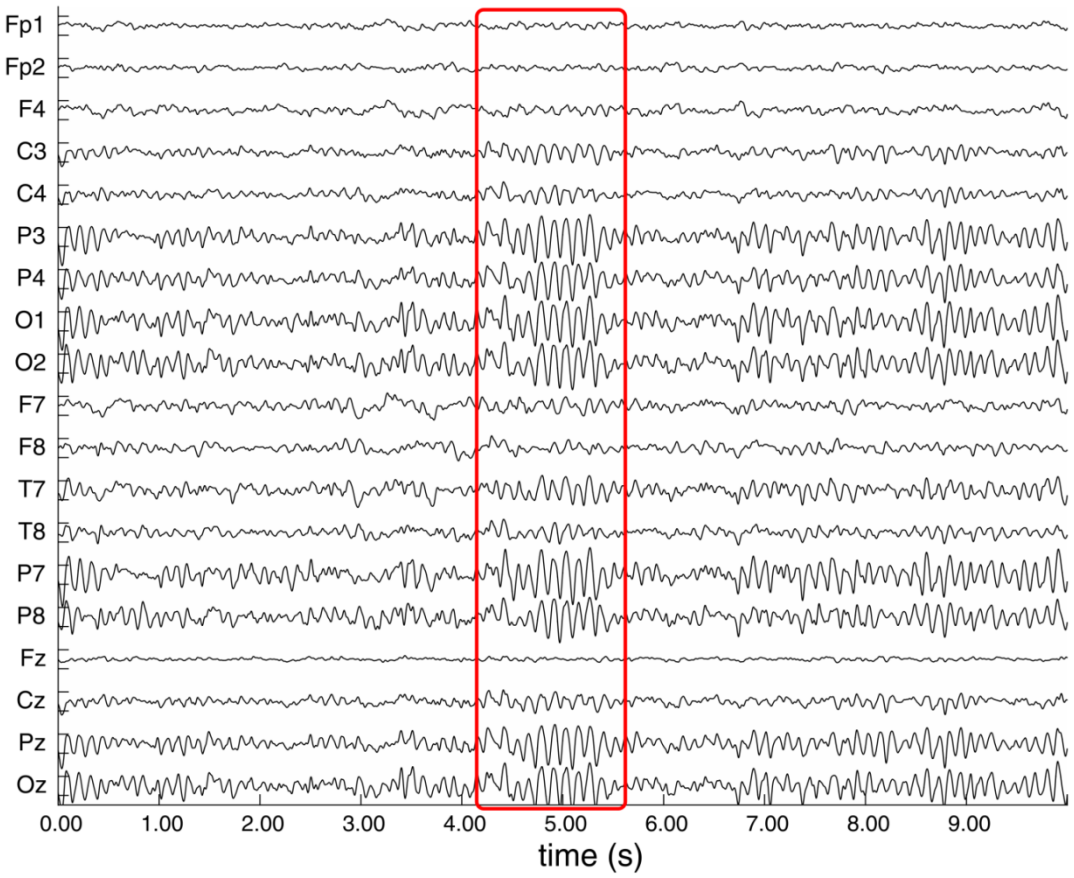

Figure 1.1: Typical EEG alpha activity

Red box highlights a period of high alpha amplitude. Alpha amplitude increases from *F*rontal towards *P*osterior/*O*ccipital leads. This example also shows the characteristic waxing and waning pattern of alpha activity over time.

Source localisation of posterior α-activity as measured by MEG indicates that these rhythms originate from regional neuronal clusters localised in the parieto-occipital cortices (Salenius, Kajola, Thompson, Kosslyn, & Hari, 1995; Salmelin & Hari, 1994; Thut, Veniero, et al., 2011; Tuladhar et al., 2007). In addition, such α-generators can be located in different cortical layers (Bollimunta, Mo, Schroeder, & Ding, 2011). Invasive recordings suggest that such sources may consist of neuronal groups with dynamically changing boundaries whose members drift in and out of synchrony, thus forming distributed cortical epicentres (Nunez, Wingeier, & Silberstein, 2001). If the population of neurons oscillating coherently at any one time becomes large enough through mutual interactions, their rhythm becomes visible as a global feature in non-invasive recordings. Such interactions may be depending on task demands. Besides intra-cortical sources, activity in thalamo-cortical neural feedback circuits appears to be involved in shaping α-rhythmicity (Hughes et al., 2011; Lopes da Silva, Vos,

Mooibroek, & Van Rotterdam, 1980; Lőrincz, Kékesi, Juhász, Crunelli, & Hughes, 2009).

Because of alpha's high visibility and distinct pattern in the human EEG, many researchers have tried to elucidate its functional significance since Hans Berger's initial description (Berger, 1929). Many theories have been proposed and tested, and many have subsequently been discarded. Alpha has been linked to many cognitive processes including, but not limited to, (working) memory (Bonnefond & Jensen, 2013; Freunberger, Werkle-Bergner, Griesmayr, Lindenberger, & Klimesch, 2011; Klimesch, Freunberger, & Sauseng, 2010), intelligence (Doppelmayr et al., 2005; Doppelmayr, Klimesch, Stadler, Pöllhuber, & Heine, 2002), oculomotor control (Mulholland & Peper, 1971; Wertheim, 1974), arousal (Makeig & Jung, 1995), attention (Sauseng et al., 2005; Thut, Nietzel, Brandt, & Pascual-Leone, 2006; Worden, Foxe, Wang, & Simpson, 2000), creativity (Lustenberger, Boyle, Foulser, Mellin, & Fröhlich, 2015), visual imagery, intentionality and motor preparation, personality differences, mental time keeping, and conscious awareness (see Shaw, 2003, for a comprehensive review over early ideas and research on α-rhythms that is beyond the scope of this dissertation).

The pervasive findings of cognitive performance that covaries with α-amplitude, phase, and/or topography suggest that alpha oscillations play a global functional role in information processing, rather than being a correlate of a limited set of specific mental processes. This is reflected by contemporary theories that assign a more general role. The putative mechanism is that α-oscillations implement selective information flow in the brain through what has been dubbed "pulsed inhibition" (Mathewson, Gratton, Fabiani, & Beck, 2009) under top-down control. "Pulsed" in this context refers to the alternating peaks and troughs of the alpha waveform, which correspond to so-called up-states and down-states of neuronal excitability. Originally used in the context of bistable membrane properties of single neurons, these states are periods within an oscillatory cycle in which the participating neurons are depolarised (that is, excitable) or hyperpolarised (less excitable or inhibited). Regional fluctuations in α-power and phase (e.g., through top down attentional control) are thought to determine the extent of local active neuronal information processing (gating by

inhibition hypothesis) (Jensen & Mazaheri, 2010) and to facilitate communication within distributed neuronal networks and between functionally connected brain areas (inhibition-timing hypothesis) (Klimesch, Sauseng, & Hanslmayr, 2007).

In the gating by inhibition framework, which rests on the finding that alpha and gamma activity are inversely correlated (Osipova, Hermes, & Jensen, 2008; Voytek et al., 2010), γ-power – and by inference neural processing – is low when α-power is high, and vice versa. Accordingly, it is hypothesised that the α-cycle, by effectively limiting the amount of information represented by γ-oscillations "nested" in its trough, acts as a salience filter which will allow only the processing of the more conspicuous or behaviourally relevant input (Jensen, Bonnefond, & VanRullen, 2012). Thus, α-activity acts as a filter by allowing only certain sensory information to be processed and communicated to downstream areas while blocking irrelevant distracters. Filtering will be stricter the higher the alpha power, that is the more neurons are engaged. The inhibition-timing hypothesis follows similar ideas but emphasises that the precise timing of activity between brain areas is important for efficient information processing, and that coordinated α-oscillations ensure that the phases within and between cooperating networks are optimally aligned. Conversely, areas with temporally non-coherent activity are prevented to communicate to reduce crosstalk. A related group of ideas considers oscillatory activity in terms of rhythmic perceptual sampling. Here, perception is thought to be a series of discrete snapshots (as compared to a continuum)(Busch & VanRullen, 2010; Schroeder & Lakatos, 2009; VanRullen & Koch, 2003; Varela, Toro, Roy John, & Schwartz, 1981), where the exact frequency (including but not necessarily limited to the 8 - 12 Hz range) determines the sampling rate, and the phase the moment when information is sampled. As such, this view stresses active information flow rather than its blocking but is effectively similar in proposing a regulatory effect through alternating time windows that either allow or restrict information processing.

These different hypotheses are not mutually exclusive, and empirical observations generally support an inhibitory, "shutter"-like role of α-activity which modulates perceptual thresholds and imposes rhythmicity into perceptual performance (e.g., Bonnefond & Jensen, 2012; Dugué, Marque, & VanRullen,

2011; Kelly, Lalor, Reilly, & Foxe, 2006; Mathewson et al., 2011, 2009; Rihs, Michel, & Thut, 2007; Thut et al., 2006).

In Experiment 1 and 2 (which are described in Chapters 2 and 3, respectively), an agnostic stance towards the functional role of alpha is assumed. In other words, we simply describe a system response that is non-informative with respect to behavioural consequences. In Experiment 3 (described in Chapter 4), the hypotheses about tACS-induced behavioural changes are based on the above presented mechanisms of sensory gating, timed inhibition, and periodic sampling, where the local magnitude and phase of α-power determine whether and to what extent visual information is processed.

Now that we have defined our target, we will have a closer look at the method by which we aim to interact with the α-rhythm. The next section gives a brief introduction of transcranial electrical stimulation methods in general and of tACS in particular, including an overview of the technical parameters that need to be considered during experimental design.

What is tACS?

Transcranial alternating current stimulation (tACS), transcranial direct current stimulation (tDCS), and transcranial random noise stimulation (tRNS) form the group of transcranial electrical stimulation (tES) methods (Bikson, Reato, & Rahman, 2013; Paulus, 2011; Woods et al., 2016). These techniques act on the stimulated tissue by inducing a subthreshold polarization which does not trigger action potentials directly, but rather changes the resting membrane potential and thus leads to a change in the firing rate or pattern of the stimulated neurons. These minimally invasive electrical brain stimulation protocols use the same hardware and all involve the application of a weak electric current of typically less than ±2 mA between two or more electrodes attached to the scalp. Their spatial specificity is in the range of centimetres, although some focality can be gained by using smaller electrodes or ring montages that increase the current density below the electrode over the area of interest. The differences between tDCS, tACS, and tRNS are in their respective current waveforms, which appear to induce different neural effects.

tDCS

tDCS is the most established form of tES in cognitive and clinical neuroscience research. As the name implies, the current waveform is time-invariant, i.e., the intensity and polarity remain constant for the duration of the stimulation. Depending on the polarity of the current, each member of an electrode pair acts either as anode (with current flowing inward towards the electrode) or cathode (current flowing outwards towards the brain). As a rule of thumb, it is often generalised that anodal stimulation leads to enhanced excitability of the underlying neural tissue, while cathodal stimulation has an inhibitory effect on the targeted brain regions (e.g., Nitsche & Paulus, 2000). In practice, polarisation likely affects different cell compartments differently, depends on the cell's orientation and depth, and will be a complex function of network connectivity (Bikson et al., 2013). Nonetheless, at least for stimulation of the motor cortex, this simplification seems to provide a reasonable working hypothesis (Jacobson, Koslowsky, & Lavidor, 2012).

It should be noted that in any tES montage, all electrodes are active in the sense that they assert an effect on the underlying tissue. An ensuing complication is that any effect ascribed to tDCS can be due to the anodal stimulation at one or cathodal stimulation at another electrode, or an interaction thereof mediated by the current that passes through the tissue between them. Extracephalic reference electrodes (e.g., on the neck or shoulder) can alleviate this problem somewhat, as can relatively smaller active electrodes over targeted areas in combination with larger "return" electrodes.

tACS

tACS and tDCS are similar in many respects in that applications typically employ similar current strength and montages, and in that they do not elicit action potentials directly but alter the membrane potential and therefore the probability for such events to occur. In contrast to tDCS, tACS involves a current waveform with periodically changing direction, that is, the polarity at each electrode reverses between anodal and cathodal at one specific frequency. Typically the tACS waveform is sinusoidal, but other waveforms are possible, e.g., saw tooth, or boxcar -shaped. tRNS can be considered a special form of

tACS in which the frequency and amplitude of the alternating current are changing randomly within a limited but broad frequency band (although the effects of tRNS resemble more those of DC stimulation reflecting changes in excitability; Chaieb, Paulus, & Antal, 2011). A tACS protocol is defined by a number of parameters, which are listed below.

Current strength

In human research the current is typically less than 3 mA from peak to trough (peak to peak amplitude). Modern stimulators have constant current control, adjusting the applied voltage with changing scalp resistance.

Montage

Montage refers to the number, location, and relative orientation of electrodes on the scalp. Electrodes can either all be placed on the scalp, or one can place an extracranial reference electrode, e.g., on the shoulder, in order to minimize the number of active electrodes over brain areas. The montage also determines the current flow between electrodes, which in turn determines which brain structures are maximally stimulated (Neuling, Wagner, Wolters, Zaehle, & Herrmann, 2012). In addition, the montage determines the degree of retinal stimulation (Laakso & Hirata, 2013) and shunting of electric current through the skin (Faria, Hallett, & Miranda, 2012; Miranda, Lomarev, & Hallett, 2006).

Electrode type/size/shape/orientation

Stimulation can be done via standard EEG electrodes or rubber electrodes in a variety of shapes and sizes. To reduce resistance between electrode and scalp, rubber electrodes are either inserted into sponges soaked in saline solution and attached using rubber bands, or covered with suitable electrode paste that also acts as glue. Smaller electrodes have a greater current density at the same current strength and are presumably more focal. Electric field models suggest that the strongest current density is typically found along the edges of the electrode and under the connector, and the field strength in the cortex is greater when connectors are positioned furthest apart (Miranda et al., 2006; Saturnino, Antunes, & Thielscher, 2015). Recent invasive recordings of tES-

induced electric fields performed in pre-surgical epilepsy patients and cebus monkeys confirmed that field strength is highest near the stimulating electrode (Opitz et al., 2016).

Frequency

The number of full positive-to-negative cycles per second, which is typically set to match a physiological frequency (that is, delta, theta, alpha, beta, or gamma; see section *The alpha rhythm,* this chapter) that has been associated with some cognitive function or state previously through correlative studies.

Phase

Stimulation waveforms can be in phase or in anti-phase across electrodes or electrode pairs. With montages of two electrodes, stimulation is always in anti-phase, i.e., when the current is positive under one electrode, it will be negative under the other. In montages with more than two electrodes, the montage can be set up in a way that the current waveforms are in phase, i.e., simultaneously either positive or negative between any given pair of electrodes. The relative phase between electrodes is thought to have either facilitatory or disrupting effect on coherence between areas (Helfrich, Knepper, et al., 2014; Polanía, Nitsche, Korman, Batsikadze, & Paulus, 2012). In experiments involving stimulus presentation, phase can also describe where in the oscillatory cycle a stimulus has been presented. The relative phase in both intrinsic and artificially induced oscillations has been linked to differences in trial-by-trial perception thresholds (e.g., Busch, Dubois, & VanRullen, 2009; Gundlach, Müller, Nierhaus, Villringer, & Sehm, 2016; Hanslmayr, Volberg, Wimber, Dalal, & Greenlee, 2013; Neuling, Rach, Wagner, Wolters, & Herrmann, 2012; Riecke, Formisano, Herrmann, & Sack, 2015; Romei, Gross, & Thut, 2012; VanRullen, Busch, Drewes, & Dubois, 2011). Intracranial measurements suggest that there is only small phase distortion between the stimulating electrode and remote electrodes, such that the phase of the stimulating waveform is representative for the overall electric field (Opitz et al., 2016). This permits assumptions about a stable phase relationship between the induced current and its spatially distributed effects.

otDCS/tSOS

If a direct current (DC) offset is applied to a tACS current, essentially creating a tAC/DCS hybrid, the stimulation can be referred to as oscillatory tDCS (otDCS). This can be hypothetically useful if one wants to combine the excitability-changing properties ascribed to DC stimulation with the oscillatory properties of tACS. This approach has been used most prominently to induce slow oscillations (< 1 Hz; then also referred to as transcranial slow oscillatory stimulation – tSOS; see section *Behavioural effects of tACS*, this chapter). During otDCS, the intensity of the current is modulated up and down in a periodic fashion but with constant polarity.

Putative mechanism of action of tACS

The periodic polarity reversal of the tACS current presumably leads to alternating hyper- and depolarisation of neuronal membranes, thereby shaping the firing rate and pattern of action potentials and thus imposing a temporal structure on neural communication (Fröhlich & McCormick, 2010). Importantly, no net polarisation builds up over time and therefore any tACS-induced effect cannot simply be explained by a modulation of the net level of excitability. Instead, tACS is thought to exert its effects through entrainment, or phase alignment, of endogenous neural activity to the phase of the electric current (see section *Entrainment: Definitions and assumptions* below). The efficacy of tACS in inducing a neural effect is thought to depend on the matching of the stimulation frequency to that of the underlying endogenous network frequency (Ali, Sellers, & Fröhlich, 2013; Schmidt, Iyengar, Foulser, Boyle, & Fröhlich, 2014), which evokes the intriguing possibility that stimulation at frequencies tuned to intrinsic spontaneous or task-related frequencies of the cortex provide a window for interaction with ongoing brain activity (Thut, Schyns, & Gross, 2011). An important prediction based on such frequency–specific interactions is that the neural effect is realised within a limited set of frequencies, including the stimulation frequency and its harmonics and subharmonics, or frequencies with a known functional relationship to the stimulation frequency, while having little to no effects at frequencies outside this defined range.

The following paragraph introduces the theoretical concept of entrainment in greater detail, before we look at experimental evidence from network simulations, in vivo and in vitro animal studies, and non-invasive research in humans that support the idea of tACS-induced entrainment.

Entrainment: Definitions and assumptions

"Neural entrainment" is one of the buzz words in contemporary neuroscience, and different researchers may use the term in a variety of ways. In the context of this dissertation, entrainment is defined as *the temporal alignment of the activity of an autonomous self-sustained neural oscillator to an externally applied weak periodic force* (Thut, Schyns, et al., 2011). This strong definition requires a neural population capable of (actively) producing rhythmic activity, and phase alignment of this intrinsic activity to the rhythm of an external driving source. This source, or *externally applied periodic force,* can be rhythmic sensory input (e.g., discrete visual, auditory, or haptic events) or, as suggested by empirical observations which are reviewed below, electromagnetic stimulation methods such as tACS, otDCS, or repetitive/rhythmic transcranial magnetic stimulation (rTMS).

The *autonomous self-sustained neural oscillator* in question is assumed to be an array, or network, of neurons that is capable, or predisposed, to oscillate at a certain intrinsic frequency (its Eigenfrequency). By "oscillate" I mean the periodic fluctuation between two states, which can be, depending on the specific context, a series of cycles from depolarisation to hyperpolarisation of a neural membrane, or the fluctuation between negative and positive scalp potentials as recorded by M/EEG. In the current work, the term oscillator refers to the neural networks supporting posterior EEG alpha rhythms (see section *The alpha rhythm* above). This network is autonomous in that it can produce and sustain rhythmic activity in the absence of external stimulation (as reflected by "spontaneous" bursts of alpha activity which are frequently observable in EEG deflections). This sort of autonomy distinguishes this type of oscillation from a (passive) resonance phenomenon that requires energy input from an external source. In this view, oscillations can serve an active, causal role in the nervous system.

The concept of a neural oscillator should be considered a simplified model. As the alpha rhythm waxes and wanes over time, it is by no means constant (sustained) over periods longer than a few seconds. As we saw earlier, it is also unlikely that there is only one single oscillator or group of homogenous oscillators. For this work it will however be assumed that, as a group, these alpha oscillators are affected by stimulation in a qualitatively similar fashion, although not necessarily to the same degree dependent on their depth and location and exact intrinsic frequency.

Fundamental theoretical considerations regarding the synchronisation of two weakly coupled oscillating systems (which are comprehensively described by (Pikovsky, Rosenblum, & Kurths, 2001) specify the response characteristics that should be exhibited by the neural oscillator to an external rhythmic force in order to qualify as entrainment as defined above. Imagine a neural array which tends to oscillate at an intrinsic natural frequency F_N, (e.g., 10 Hz for the alpha network) and an external periodic force with stimulation frequency F_S (e.g., tACS). The efficiency by which the periodic force can entrain (or phase-align) the self-sustained oscillator depends on two factors: the distance between intrinsic and external frequency, and the intensity of the external force. For a small frequency mismatch (i.e., a small difference between F_N and F_S), only a low stimulation intensity is required to phase-lock the oscillator's activity to that of the stimulating force. When this happens, the frequency of the oscillator will be adjusted to match that of the force. For larger frequency mismatches, more energy (that is, higher stimulation intensity) is required to achieve the same degree of phase locking, and thus frequency alignment, between the two signals.

An equivalent way to look at this is that for a given moderate intensity (that is, an intensity that is neither too weak to have any influence nor too strong to overpower intrinsic rhythms), its ability to entrain the network is high when the frequencies are (near) equal but decreases when the difference between frequencies increases. If the mismatch is large and the intensity low, there may be partial entrainment, with the oscillator's frequency somewhere between its spontaneous frequency and the frequency of the external force (Fröhlich, 2015).

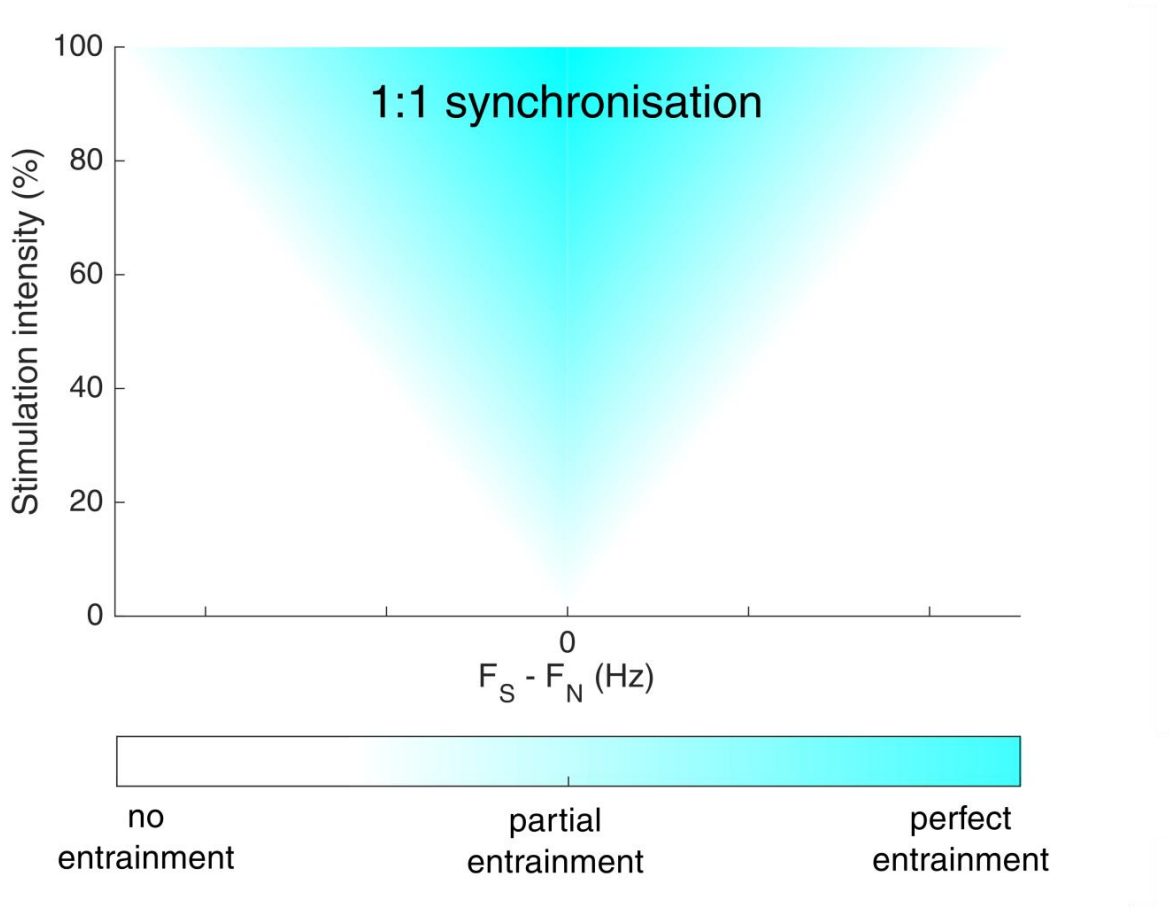

Figure 1.2: Entrainment exhibits an Arnold tongue

The extent to which an external periodic force can entrain, or phase-align, a weak neural oscillator (shaded blue area) depends both on the intensity of the external force (y-axis, arbitrary unit) and the mismatch between the intrinsic frequency of the neural oscillator, F_N, and the frequency of the external force, F_S (x-axis). At low intensities, entrainment is confined to closely matching stimulation frequencies, while at higher intensities a given network can be entrained by a wider range of F_S, thereby experiencing a frequency shift.

When the strength of entrainment is plotted as a function of stimulation intensity and frequency mismatch between F_N and F_S, one observes a characteristic triangular area called an Arnold tongue (see Figure 1.2). This Arnold tongue delimits a parameter space within which the strength of entrainment of any available oscillator is inversely proportional to the relative frequency mismatch and directly proportional to the stimulation intensity. These considerations apply also if the stimulation frequency is close to a harmonic or subharmonic of the intrinsic frequency.

Evidence for tACS-induced entrainment of neural activity

Entrainment by weak AC electric fields has been studied in silico, in vitro and in vivo. Increased coherence of spike timing with the phase of the applied field depending non-linearly on stimulation frequency and intensity have been

demonstrated in brain slice experiments (Deans, Powell, & Jefferys, 2007; Reato, Rahman, Bikson, & Parra, 2010; Schmidt et al., 2014). Notably, such changes in spike timing can occur in the absence of a net change in spike rate (Reato et al., 2010).

Enhanced phase alignment for low stimulation frequencies was also found in live, anaesthetised rats (Ozen et al., 2010). Critically, this result did not generalise to awake behaving rats, thereby emphasising the dependence of such effects on endogenous network dynamics and calling into question whether behaviour born of normal complex neural processing, or the neural activity of a system as a whole, are in principle responsive to weak interventions. More recently, an Arnold tongue has been demonstrated in a physiologically plausible neural network model, which was partly confirmed by recordings from the brains of anaesthetised ferrets (Ali et al., 2013). Taken together, these studies provide direct empirical evidence that weak external alternating electric fields can entrain the activity of neurons and neural networks. They also show that the extent to which stimulation can influence network activity depends on the interplay between the stimulation frequency and intensity, and on the underlying intrinsic network dynamics, in line with the theory of weak coupled oscillators.

Entrainment has also been studied non-invasively in humans using EEG and MEG. Initial evidence for the entrainability of the human brain comes from research on photic driving in humans (Halbleib et al., 2012; Herrmann, 2001; Notbohm, Kurths, & Herrmann, 2016), which indicates that the neural response to flickering light stimulation is strongest when the flicker frequency is at or close to the Eigenfrequency of the visual cortex. In contrast to visual stimulation, observing neural entrainment during tACS is technically much more challenging. A major caveat for recordings with MEG and EEG during electrical stimulation is the strong electromagnetic artefact that exceeds the amplitude of the signal by several orders of magnitude. In case of AC stimulation targeting intrinsic frequencies (e.g., stimulating at individual alpha frequency), this artefact has the same frequency characteristics as the signal under study. Unless the artefact can be removed perfectly (which is still a controversial question; see Noury, Hipp, & Siegel, 2016) there is a risk of interpreting systematic noise

as neural activity. Notwithstanding, parieto-occipital EEG alpha power enhancement has been demonstrated during 10 Hz tACS over the posterior brain (Helfrich, Schneider, et al., 2014). The same group also showed increased interhemispheric EEG coherence in the γ-band during 40 Hz tACS when both hemispheres were stimulated in-phase, compared to out of phase (Helfrich, Knepper, et al., 2014). To avoid the analytical and interpretational pitfalls of artefact removal, other EEG work has focused on frequency-specific enhancement beyond the stimulation artefact (aftereffects) as evidence for successful entrainment. This work will be reviewed in detail in Chapter 2 as it sets the context for Experiment 1.

MEG, especially in source space, may be less susceptible to distortion by the artefact (Neuling et al., 2015; Witkowski et al., 2016). It could be shown that different tACS-frequencies interacted differentially with the steady state evoked response induced by visual flicker, although the relationship between tACS and the neural response was more complex than a simple, homogenous entrainment account would suggest (Ruhnau, Keitel, Lithari, Weisz, & Neuling, 2016). A stronger case for entrainment was made by Ruhnau and colleagues by demonstrating increased phase coherence between the tACS waveform and occipital activity during tACS at individual alpha frequency (Ruhnau, Neuling, et al., 2016). This effect was constrained to periods where participants kept their eyes open, compared to eyes closed, once more suggesting state-dependence of the neural response. Finally, in a different approach using amplitude-modulated tACS with a carrier frequency of 220 Hz modulated at individual theta frequency to avoid the artefact in the theta range, Chander and co-workers showed both increased interhemispheric theta phase locking and theta power in their stimulation group compared to participants in the sham group, along with a decrement in working memory performance (Chander et al., 2016).

Finally, functional magnetic resonance imaging (fMRI) data may provide indirect evidence that tACS can entrain alpha networks. Decreased metabolic activity in response to visual targets has been demonstrated during α-tACS in brain areas for which a negative correlation between the blood oxygenation level dependent (BOLD) response and α-amplitude had been shown previously (Vosskuhl, Huster, & Herrmann, 2016). While in a series of fMRI experiments

Cabral-Calderin and colleagues did not observe these specific changes (under different conditions and using a different montage), these researchers concluded that the BOLD modulation by tACS depends on the combination of task and tACS frequency. As the topography of their effects was poorly predicted by either task or electrode position they also suggested that this might reflect different degrees of entrainability of the stimulated brain regions (Cabral-Calderin, Weinrich, et al., 2016). This group later went on to show that resting state functional connectivity was affected in a frequency-specific manner, with 10 Hz and 40 Hz tACS resulting in increased and decreased connectivity, respectively (Cabral-Calderin, Williams, Opitz, Dechent, & Wilke, 2016). Such frequency-specific connectivity changes could possibly explain some of the complex distal metabolic effects induced by different tACS frequencies in their earlier study.

To sum up, there is empirical support for entrainment of neural network activity by tACS both from fundamental and human neuroscience. The latter is unavoidably less direct and more prone to electromagnetic distortion and, by extension, interpretational error. Partly for this reason, little research in humans has focused specifically on describing the neural mechanisms by which tACS can change behaviour, and more research has focused on the behavioural changes as such. This research is reviewed in the next section.

Behavioural effects of tACS

A range of studies employing different stimulation parameters, tasks, and outcome variables provide evidence for frequency- and/or phase specificity of tACS-induced effects on overt behaviour.

tACS of the motor system

A comparatively large number of studies have dealt with stimulation of the motor cortex, the effects of which are either probed by inducing motor evoked potentials (MEP) using single TMS pulses to the motor area, or by having participants perform simple motor tasks. Motor function is generally associated with periodicity in the beta range (e.g., Hari & Salmelin, 1997). Consistent with this well-known association, some of these studies show that tACS in the beta range (typically 20 Hz) is accompanied by increased MEP amplitude as an

indication of greater cortical excitability (Feurra et al., 2013; Feurra, Bianco, et al., 2011; Schutter & Hortensius, 2011), and by a slowing of voluntary movement (Joundi, Jenkinson, Brittain, Aziz, & Brown, 2012; Pogosyan, Gaynor, Eusebio, & Brown, 2009; Wach et al., 2013a). This suggests an interaction between 20 Hz-tACS and intrinsic oscillations in the beta range. However, more complex interactions than simple phase entrainment most likely play a role, as the effect depends at least to some extent on the motor state of the participant (task or rest; Feurra et al., 2013), and on when the dependent measures are acquired (e.g., Wach et al., 2013a, found no MEP changes offline after 20 Hz stimulation). In addition, changes in motor output have also been observed after tACS at higher (Joundi et al., 2012; Moliadze, Antal, & Paulus, 2010) and lower frequencies (Feurra et al., 2013; Zaghi et al., 2010). To complicate matters further, the net effect of tACS may be non-linearly dependent on the intensity of stimulation (Moliadze, Atalay, Antal, & Paulus, 2012). Finally, MEG data suggest that tACS at one frequency can induce cross-frequency changes (Wach et al., 2013b).

These results show that overall tACS appears to be effective in modulating motor-related activity, although the net effect depends on a number of factors and may be hard to predict based on the protocol alone. The fact that these studies variably test motor output either during or after tACS, and apply tACS during rest or during task performance, hinders comparability but the repeated findings of lasting changes in the motor response after stimulation has ended suggest that the latter are at least partly supported by plastic changes.

tACS in perception

In the domain of perception, stimulation of the visual cortex has focused mostly on the attempt to induce alpha or gamma oscillations, whose respective roles in vision are often dichotomised as distracter suppression versus stimulus processing, respectively (e.g., Bonnefond & Jensen, 2013) and which have been found to modulate one another (Jiang, Bahramisharif, van Gerven, & Jensen, 2015; Osipova et al., 2008; Voytek et al., 2010). Frequency-specific changes in visual task performance after γ-tACS at 60 Hz have been reported in the form of improved contrast discrimination (Laczó, Antal, Niebergall, Treue, & Paulus, 2012) and reduced perceptual stability under bistable viewing conditions

(Cabral-Calderin, Schmidt-Samoa, & Wilke, 2015). In addition, 40 Hz tACS delivered either in-phase or out-of-phase between hemispheres was effective at changing apparent motion direction differentially while also modulating interhemispheric EEG coherence, which was taken as evidence for a causal role of 40 Hz rhythmicity in perceptual binding (Helfrich, Knepper, et al., 2014).

Alpha stimulation has been found to modulate the sound-induced double flash illusion in a manner consistent with alpha-frequency dependent cyclic sampling of visual information (Cecere, Rees, & Romei, 2015; VanRullen & Koch, 2003). In addition, accuracy in a visual oddball task was found to be modulated by tACS phase, although the specificity of this result to alpha is uncertain as no control frequency was included (Helfrich, Schneider, et al., 2014). In a different line of research, a somewhat mixed result was reported by Brignani and colleagues (Brignani, Ruzzoli, Mauri, & Miniussi, 2013). In their study, tACS was laterally applied over visual cortex to modulate visuospatial attention. tACS-induced changes in the performance of a visual detection task were only moderately frequency-specific to lower (at and below alpha) frequencies and did not exhibit the spatial profile that was predicted based on the common observation of alpha lateralisation in spatial attention tasks. Experiment 3 of this dissertation is based on this study, which will be reviewed in more detail in Chapter 4.

In the auditory domain, the phase of both 4 Hz and 10 Hz alternating currents was shown to affect auditory detection performance (Neuling, Rach, et al., 2012; Riecke, Formisano, et al., 2015; Riecke, Sack, & Schroeder, 2015), again in line with an entrainment account. Finally, tACS over somatosensory cortex in the alpha and high γ-range was found to elicit stronger tactile sensations in the contralateral hand than stimulation at other frequencies (Feurra, Paulus, Walsh, & Kanai, 2011). Alpha-tACS also modulated tactile detection thresholds in a phase-specific manner (Gundlach et al., 2016), mirroring the findings in the visual and auditory domain.

tACS and tSOS in the cognitive domain

Intriguingly, periodic stimulation also appears to be effective in modulating oscillations implicated in higher order cognition, including logical

reasoning (Santarnecchi et al., 2013, 2016), decision making (Sela, Kilim, & Lavidor, 2012), creativity (Lustenberger et al., 2015), facial emotion perception (Janik, Rezlescu, & Banissy, 2015), working memory (Jaušovec & Jaušovec, 2014; Meiron & Lavidor, 2014; Pahor & Jaušovec, 2014), and short-term memory (Feurra, Galli, Pavone, Rossi, & Rossi, 2016; Polanía et al., 2012; Vosskuhl, Huster, & Herrmann, 2015). While overall, most of these reports need independent confirmation, research across different laboratories has focused on the use of tSOS (see section *What is tACS?* above) to enhance memory performance. Studies involving frontal stimulation at slow (< 1 Hz) frequencies were able to show an improvement of declarative memory (Antonenko, Diekelmann, Olsen, Born, & Mölle, 2013; Kirov, Weiss, Siebner, Born, & Marshall, 2009; Marshall, Helgadóttir, Mölle, & Born, 2006), in line with the putative role of slow wave sleep for memory consolidation (Rasch & Born, 2013) and the notion of slow wave entrainment. Such stimulation particularly during sleep was sometimes accompanied by changes in EEG slow wave and spindle activity following stimulation (Marshall et al., 2006; Paßmann et al., 2016; Sahlem et al., 2015) in addition to long term modulatory effects on sleep homeostasis (Reato, Gasca, et al., 2013).

Attempts to replicate this memory enhancement have been mixed. While some studies failed to find evidence for improvement (Eggert et al., 2013; Sahlem et al., 2015), others even reported negative effects (Garside, Arizpe, Lau, Goh, & Walsh, 2015; Paßmann et al., 2016). Possible reasons for these different outcomes may be found in the different population characteristics (for instance, Eggert et al., 2013, and Paßmann et al., 2016, tested older participants), on whether both hemispheres are stimulated in-phase or in anti-phase (Garside et al., 2015, used standard anti-phase frontal tACS as compared to the bilateral in-phase tSOS montages typically applied in the other experiments), as well as brain state (Kirov et al., 2009; Marshall et al., 2006).

Different approaches with frequencies other than slow oscillations have been successfully employed to improve memory. After tACS at 140 Hz was applied in-phase to bilateral frontal cortices as a proxy of hippocampal ripple waves, participants showed less overnight forgetting of word-pairs compared to sham (Ambrus et al., 2015). In an interesting recent experiment, ongoing EEG

was monitored in real-time to trigger tACS at spindle frequency (12 Hz) during endogenous sleep spindle activity in order to amplify, rather than impose, a specific brain state (Lustenberger et al., 2016). This intervention resulted in enhanced spindle activity during the period after stimulation and this change was correlated with better retention of a finger tapping task.

In sum, memory appears to be a cognitive construct amenable to stimulation with tACS or tSOS at different frequencies, but a number of factors are likely to determine the outcome that still need further exploration. While tACS has also been used successfully in the modulation of other cognitive functions, more data needs to be acquired before strong conclusions about the interaction between the current and task-relevant intrinsic oscillations, and by extension a causal role of the latter, can be made.

tACS in clinical applications

A few experiments have directly looked at the utility of tACS as a therapeutic device. Aberrant alpha activity has been implicated to play a role in tinnitus. However, neither frontal nor temporal alpha-tACS was particularly effective in reducing tinnitus loudness or distress ratings (Vanneste, Fregni, & De Ridder, 2013; Vanneste, Walsh, Van De Heyning, & De Ridder, 2013). In patients with mild Parkinson's disease, frontal tACS at 77.5 Hz in a longitudinal between subject design was unable to improve PD or psychological symptoms compared to sham after 10 treatment sessions (Shill, Obradov, Katsnelson, & Pizinger, 2011) and, ironically, resulted in one patient's increase in tinnitus. On the other hand, 20 Hz tACS over motor cortex differentially affected cortico-muscular coherence and motor performance in PD patients (compared to sham and 10 Hz tACS) but not healthy controls (Krause et al., 2014). This implicates once more that the effect of tACS may depend on the population and possibly, the integrity of the neural network stimulated.

An intriguing observation in the context of PD treatment, and simultaneously a strong case for direct (phase) interaction with endogenous rhythms, comes from a set of studies on muscle tremor in Parkinson patients (Brittain, Probert-Smith, Aziz, & Brown, 2013) and healthy participants (Mehta, Brittain, & Brown, 2014; Mehta, Pogosyan, Brown, & Brittain, 2014). In the

former study, it was shown that pathological tremor was either enhanced or reduced by stimulation of the motor cortex at tremor frequency, depending on the phase difference between the tACS current waveform and the time course of the tremor. Strikingly, tremor could be significantly reduced when the tACS phase was adjusted online to account for fluctuations in tremor frequency, demonstrating that tACS could essentially cancel out the endogenous oscillation in a phase specific manner. In healthy subjects, it was confirmed that this tACS protocol was effective in promoting phase alignment of physiological tremor (Mehta, Brittain, et al., 2014; Mehta, Pogosyan, et al., 2014), thereby supporting the entrainment hypothesis of tACS. Gamma-band tACS was recently tested in minimally conscious patients and healthy controls as a tool to increase brain connectivity and assess neural responsivity (Naro, Bramanti, Leo, Russo, & Calabrò, 2016). While there were differential changes in power and coherence measures between healthy controls and different patient subgroups, tACS did not manage to alleviate symptoms according to the Glasgow Recovery Coma Scale.

Although overall these results are not overwhelming, the lack of large-scale and completed clinical trials means that it is far too early to abandon hope for tACS as a treatment. Of note, despite the prospect of interacting directly with pathological oscillations, at the time of writing, a keyword search for "transcranial alternating current stimulation" on the World Health Organisation's website for the International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/) returned only five registered clinical trials, underlining the need for more research in this area.

In sum, there is a wide range of potential applications for tACS, and the current body of literature abounds with findings that are worth following up. Although at this stage the evidence for specific interactions can only be considered preliminary due to the rather anecdotal nature of individual observations, there are good reasons to believe that tACS can modulate neural activity and behaviour via entrainment of endogenous oscillations. However, as will be reviewed in detail in the next chapter, observations of (particularly neural) changes that persist long after stimulation has been switched off strongly suggest that active phase alignment of intrinsic neural rhythms to ongoing tACS

might be only part of the story. In order to explain these lasting aftereffects, some mechanism must be exist that can maintain altered network dynamics in the absence of a perpetually rhythmic supply of energy. Theoretically, phase alignment could be maintained for a period of time by intrinsic dynamics, much like a pendulum will continue to swing for a little while (albeit with decreasing amplitude) when the external drive has been removed. As we will see, this scenario is physiologically implausible. Therefore, such a mechanism likely involves some form of structural neural plasticity, for example by changing synaptic efficacy or by selectively strengthening or weakening neural connectivity depending on their susceptibility to the induced electric fields.

The next three chapters describe experimental work conducted for this dissertation that attempts to disentangle the relative contribution of online entrainment to concurrent behaviour and offline neural aftereffects. Each chapter includes a review of relevant studies that have not been considered in the introduction to avoid repetition. Chapter 2 and 3 follow up on studies investigating the aftereffects of tACS (in particular, aftereffects on endogenous alpha activity in the posterior cortex following stimulation with tACS at alpha frequency). Specifically, Chapter 2 compares the response characteristics of the alpha aftereffect with those predicted by the entrainment account. Chapter 3 investigates whether the magnitude of the alpha aftereffect conforms to predictions of a spike-timing dependent plasticity model. Moving to online effects, Chapter 4 investigates the phase- and frequency-dependence of tACS on visuospatial attention, operationally defined as visual detection of peri-threshold stimuli, during stimulation.

Thesis at a glance (Abstracts)

Experiment 1: Alpha power increase after transcranial alternating current stimulation at alpha-frequency (α tACS) reflects plastic changes rather than entrainment

Periodic stimulation of occipital areas using tACS at α-frequency (8 - 12 Hz) enhances EEG α-oscillation long after tACS-offset. Two mechanisms have been suggested to underlie these tACS-induced changes in oscillatory EEG activity: entrainment of brain oscillations and/or changes in oscillatory circuits

by spike timing-dependent plasticity. We tested to what extent plasticity can account for tACS-aftereffects when controlling for entrainment "echoes". To this end, we used a novel, intermittent tACS protocol and investigated the strength of the aftereffect as a function of phase continuity between successive tACS episodes, as well as the match between stimulation frequency and endogenous α-frequency. Twelve healthy participants were stimulated at individual α-frequency (IAF) for 15 - 20 min in four sessions using intermittent tACS or sham. Successive tACS events were either phase-continuous or phase-discontinuous, and either 30 or 80 α-cycles long. EEG α-phase and power changes were compared after and between episodes of α-tACS across conditions and against sham. Alpha aftereffects were successfully replicated after intermittent stimulation using 80-cycle but not 30-cycle trains. These aftereffects did not exhibit any of the characteristics of entrainment echoes in that they were independent of tACS phase-continuity and showed neither prolonged phase alignment nor frequency synchronisation to the exact stimulation frequency. These results indicate that entrainment is insufficient and additional mechanisms such as plasticity are necessary to explain α-aftereffects in response to α-tACS.

Experiment 2: Aftereffects are not replicated testing the spike timing-dependent plasticity hypothesis of α-tACS-induced alpha power enhancement

Experiment 1 indicated that stimulation at slightly lower than the endogenous α-network frequency leads to α-power enhancement, and that such α-aftereffects might arise through plastic changes. Based on a conceptual model of spike timing dependent plasticity that has been proposed to explain these changes, we tested whether it is possible to induce directional changes in dependence of the relative mismatch of the tACS frequency to the IAF. We stimulated fourteen (different) healthy participants on different days with an eight second intermittent tACS protocol (similar to the 80-cycle protocol of Experiment 1) at 0.75 Hz above or below their IAF. As control conditions, participants also underwent a session with continuous stimulation and a sham session. We did not observe the predicted α-power change observed in the previous experiment in any of the active conditions. The discrepancy between the two results under very similar conditions is discussed, and problems in the

experimental design and analysis are scrutinised. The lack of consistency between the two experiments raises concerns regarding the reproducibility and effect size of tES effects.

Experiment 3: No evidence for a role of alpha entrainment in visuospatial bias induction when lateralised α-tACS is applied to the right occipito-parietal cortex (Experiment 3)

Hemispherically lateralised α-tACS has been studied previously for its ability to induce a visuospatial attentional bias away from the stimulated hemisphere (Brignani et al., 2013), according to the putative role of α-activity in attentional gating. The results of this study were not strongly suggestive of α-entrainment but were limited by a number of problems in the experimental design. In the current experiment, some of these design issues were addressed to increase the likelihood to observe entrainment and associated changes in attentional bias. Twenty healthy participants performed a peripheral visual dot detection task with target size titrated to near each individual's detection threshold. tACS was applied in two thirds of the trials at either IAF (Alpha) or 40 Hz (Gamma) over the right parieto-occipital cortex in an event-related design. Compared to trials without tACS, accuracy was slightly lower on average for targets presented in the left visual hemifield in both Alpha and Gamma trials. However, these results were not statistically significant. No difference between tACS conditions was found for right-sided or bilateral targets. While lateralised tACS might have a negative effect on detection accuracy in the contralateral visual field, this effect is neither frequency-specific nor waveform phase-dependent. Problems with this interpretation are discussed.

Chapter 2. Alpha power increase after transcranial alternating current stimulation at alpha-frequency (α-tACS) reflects plastic changes rather than entrainment (Experiment 1)

As reviewed in the general introduction, evidence accumulates that during (i.e., online to) stimulation tACS exerts at least some of its effects through entrainment or resonance of underlying neural networks. However, the online measurement of neural responses is complicated by the massive stimulation artefact in electrophysiological recordings introduced by the current, which is several orders of magnitude larger than the signal. Aside from the technical challenge, there is great hope that tACS will prove effective in the treatment of disorders involving abnormal neural network activity such as schizophrenia (Uhlhaas & Singer, 2006). Any therapeutic application will depend crucially on the ability of tACS to stimulate network plasticity. Accordingly, another line of research has been concerned with changes in oscillatory activity that persists beyond the offset of periodic stimulation. Aftereffects have been reported after a variety of stimulation protocols in a variety of frequency bands (reviewed recently in Veniero, Vossen, Gross, & Thut, 2015). These aftereffects include (offline) changes in spectral power or measures of connectivity, which have been casually referred to as entrainment (e.g., Marshall et al., 2006; Zaehle, Rach, & Herrmann, 2010). These aftereffects are often complex, and of significantly larger duration as expected based on theoretical considerations of synchronization. The latter make strong predictions about the dynamic relationship between the entraining force and the oscillator, and it will become clear that other mechanistic explanations, such as synaptic plasticity, need to be considered to explain the empirical findings.

The next paragraph provides a short overview of studies that have reported an EEG power increase at the stimulation frequency following periodic electrical stimulation (see also Veniero et al., 2015). Although some of the research included involves the use of oscillatory tDCS rather than tACS, these studies apply similar hypotheses and are therefore relevant to this discussion.

Aftereffects were first reported and replicated after slow frequency (0.75 Hz, also referred as slow delta) stimulation over frontal areas (also called

transcranial slow oscillation stimulation/tSOS) (Antonenko et al., 2013; Eggert et al., 2013; Marshall et al., 2006; Marshall, Kirov, Brade, Mölle, & Born, 2011; Reato, Gasca, et al., 2013) aiming to either enhance or disrupt slow wave sleep, whose hallmark rhythm has been associated with memory consolidation (see e.g., Rasch & Born, 2013). Using anodal stimulation over bilateral frontal cortices (F3/F4) at 0.75 Hz during sleep, these studies showed that tSOS leads to somewhat reliable and replicable EEG effects at low frequencies (delta band, 1 - 4 Hz), as well as to a subsequent improvement in memory performance. These results have been explained by slow wave entrainment, a notion that was supported by enhanced phase continuity of the stimulation waveform after the current had subsided (Marshall et al., 2006; Reato, Gasca, et al., 2013). However, phase entrainment effects are generally expected to diffuse quickly after stimulation subsides, and these authors also found no significant phase coherence beyond twenty seconds after tSOS (Reato, Gasca, et al., 2013, their Figure S2).

In addition, delta power changes were often observed alongside changes in other, non-harmonic frequency bands, and mixed results have been reported regarding the direction of modulation (i.e., enhancement: Antonenko et al., 2013; Kirov et al., 2009; Marshall et al., 2006; versus suppression: Eggert et al., 2013), and secondary spectral changes (e.g., α-increase after tSOS during sleep: Marshall et al., 2006; versus theta increase during wakefulness: Kirov et al., 2009). This may reflect dependence of the response on population characteristics (Eggert et al., 2013), as well as on brain state (Kirov et al., 2009; Marshall et al., 2006). Taken together, these observations suggest that, other mechanisms than entrainment or resonance play a role in shaping the aftereffects of periodic electrical stimulation. This is also highlighted by the lasting impact of tSOS on subsequent sleep homeostasis (Reato, Gasca, et al., 2013) and on declarative memory long (that is, up to hours) after stimulation has ceased.

Another series of studies investigated the aftereffects of prolonged (in the range of 10 - 20 min) rhythmic electrical stimulation at α-frequencies on spectral power in the α-band (Helfrich, Schneider, et al., 2014; Neuling, Rach, & Herrmann, 2013; Neuling, Rach, et al., 2012; Zaehle et al., 2010). This type of

stimulation reliably resulted in enhanced posterior α-power, suggesting a frequency-specific interaction between tACS and the underlying cortical network activity. Zaehle and colleagues (2010) stimulated ten healthy volunteers for ten minutes over bilateral occipital cortex (PO9/PO10) at their individual alpha frequency (IAF) while they were performing a visual vigilance task. Resting EEGs were obtained before (pre-test) and after stimulation (post-test). Compared to a control group who received only sham stimulation, the tACS group showed elevated mean α-power during post-test EEG. This change was specific to the frequency band including the stimulation frequency/IAF and did not extend to neighbouring frequencies. The authors took this enhancement as support for the entrainment of alpha oscillations by tACS but also suggested spike timing-dependent plasticity (STDP) at the origin of these changes. A proof-of-principle computational STDP model relating synaptic weight changes, network frequency, and stimulation frequency demonstrated an increase in synaptic weights for neural circuits communicating through feedback loops with a frequency near that of rhythmic external stimulation and a decrease at other frequencies.

Neuling and colleagues (2012) also demonstrated α-enhancement after stimulation over bilateral temporal sites (T7/T8) with a DC current whose amplitude was modulated sinusoidally at 10 Hz. In addition, the auditory detection thresholds of their participants were dependent on the phase of the modulation during stimulation, suggesting online entrainment. As there was no control group in this study, the α-power change could not unequivocally be attributed to tACS. However, a subsequent experiment (Neuling et al., 2013) comparing two groups that received active versus sham stimulation found α-power enhancement after applying 20 min of continuous tACS at IAF with a midline Cz-Oz montage. Notably, this relative enhancement was only observed when participants kept their eyes open during the experiment, in which case the group effect lasted at least for an impressive thirty minutes. In contrast, when participants kept their eyes closed (and as a consequence α-power was generally elevated), no enhancement occurred. Again, the duration of the effect suggests a mechanism beyond entrainment. Finally, α-enhancement was reported in a within subject sham-controlled design, allowing for even stronger conclusions (Helfrich, Schneider, et al., 2014). The magnitude of the relative α-increase post

tACS was positively correlated with relative enhancement during tACS, suggesting a direct link between online entrainment and offline aftereffect.

To sum up, two main mechanisms have been suggested to explain alpha power enhancement at the stimulation frequency after α-tACS: direct entrainment of underlying brain oscillations during stimulation that might be sustained beyond the offset of tACS (as entrainment "echoes")(Antal & Paulus, 2013; Herrmann, Rach, Neuling, & Strüber, 2013; Reato, Rahman, Bikson, & Parra, 2013; Thut & Miniussi, 2009), and/or plastic changes, possibly via spike timing-dependent plasticity mechanisms (Polanía et al., 2012; Zaehle et al., 2010).

The present study set out to explore the dependence of α-power aftereffects on sustained online entrainment. We tested to what extent plasticity can account for tACS-aftereffects when controlling for entrainment echoes, i.e., entrained activity that remains stable after the end of rhythmic stimulation. To this end, we employed an intermittent tACS-protocol and applied short parieto-occipital α-tACS trains interrupted by breaks of equal duration. Total tACS-duration was comparable to the continuous α-tACS-protocols previously reported to lead to offline α-enhancement (Helfrich, Schneider, et al., 2014; Neuling et al., 2013; Neuling, Rach, et al., 2012; Zaehle et al., 2010). In order to assess the contribution of entrainment echoes to the α-aftereffect, we manipulated phase-continuity (continuous versus discontinuous) between successive α-tACS trains. Based on observations online to tACS (see Helfrich, Schneider, et al., 2014) as well as theoretical groundwork (Pikovsky et al., 2001; Zaehle et al., 2010), we reasoned that if entrainment echoes come into play, α-enhancement should be 1) stronger when intermittent α-tACS trains are applied in phase-continuous versus phase-discontinuous regimes, 2) centered at stimulation frequency rather than intrinsic Eigenfrequency, and 3) stronger when the stimulation frequency matches the spontaneous α-frequency, while 4) EEG phase-locking to the phase of the tACS-train should outlast tACS-offset as a minimum requirement for stable entrainment over minutes. Our EEG results confirmed enhanced α-power after α-tACS compared to sham stimulation in the present sample, but did not reveal any of the hypothesised offline entrainment characteristics. Consistent with plasticity as the predominant mechanism for

Table 2.1: Participant demographics and stimulation parameters.
ISF = Individual stimulation frequency; mA/pp = milliampere peak to peak.

ID	*Sex*	*Age*	*ISF*	*Intensity (mA/pp)*
01	m	25	10.0	2.00
03	f	31	10.0	1.65
04	f	26	8.0	1.60
05	m	20	10.5	2.00
06	m	26	11.0	2.00
07	f	29	11.0	2.00
12	m	22	9.5	2.00
13	m	24	11.0	1.80
15	f	37	10.0	2.00
16	m	32	9.0	2.00
17	f	24	10.0	1.75
18	f	32	11.0	1.35

aftereffects, α-enhancement 1) occurred irrespective of phase-continuity between trains, 2) was observed at spontaneous α-peak frequency, and was 3) neither stronger with tACS at intrinsic α-frequency, nor 4) associated with prolonged phase-locking beyond tACS.

Methods

Participants

Eighteen volunteers were invited to this experiment. All volunteers gave written informed consent and received monetary compensation of £9/hour for their participation. Of these, three were excluded as they showed no discernible alpha activity, while one person failed to show up. One person terminated the experiment after she suffered a panic attack that was unrelated to tACS. One person discontinued after having developed a strong discomfort to even very mild currents after two active sessions but agreed to record a tACS-free control session. Twelve healthy volunteers (six male, age 27 ± 5 years; see Table 2.1) completed all four protocols. The experiment was approved by the local ethics committee of the College of Science and Engineering (CSE01198), University of Glasgow, according to the British Psychological Society code of ethics and conduct. No participants reported a history of neurological/ psychiatric disorders or any other contraindication to tACS (current use of psychoactive

medication/drugs, metal implants, pregnancy; see Appendix A for safety questionnaire with screening questions).

Procedure

Participants were recruited from among colleagues and the university's subject pool. They received information about the experiment and a safety questionnaire to assess their suitability before they were officially invited to take part in the study. Each participant underwent four sessions of maximally two hours each. Sessions were at least three days apart to avoid carry over effects. In the first session, participants were familiarised with the equipment and the experimental procedure. The safety questionnaire had to be filled in before every session in order to ensure that participants were still fit to receive stimulation. Preparation of tACS- and EEG-electrodes took approximately 45 min. Data recording including resting EEGs and a stimulation protocol lasted approximately 40 min.

For a schematic of the setup and procedure see Figure 2.1. Data acquisition started with two minutes of resting EEG with eyes closed, and two minutes of resting EEG with eyes open while fixating on a white fixation cross on a dark-grey background (pre-test). In the first session only, the individual stimulation frequency (ISF) and intensity were then determined (for details see section *tACS* below). In every subsequent session each participant received a few trains at their ISF to make sure they would be comfortable and see no phosphenes during the protocol.

Participants then underwent one of the four stimulation protocols (described in section *tACS* below) in counterbalanced order while EEG was continuously recorded. For the duration of each protocol, participants performed a slow visual colour change detection task to ensure they stayed alert and to keep the cognitive state similar across participants.

After completion of the task and tACS protocol, an additional two minutes of resting EEG with eyes open followed by two minutes with eyes closed were recorded (post-test) as during the pre-test. At the end of each session, participants filled in a questionnaire with visual analogue scales (VAS) in which

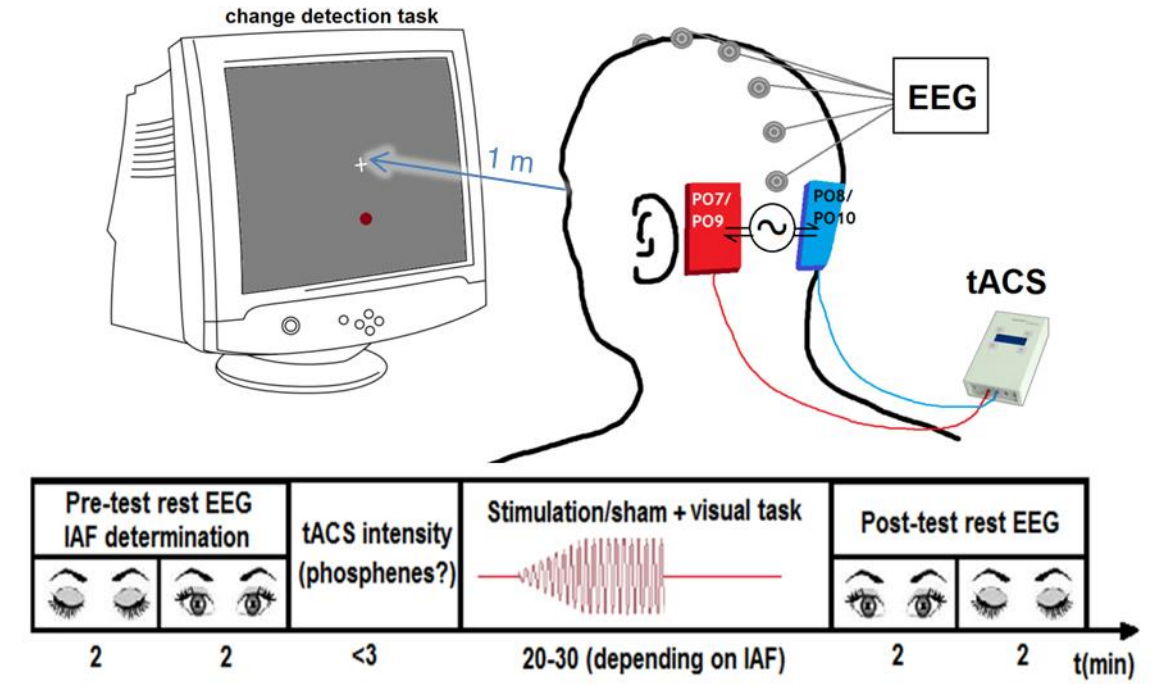

Figure 2.1: Experimental setup and procedure.
For details refer to section *Procedure*.

they rated how strongly they had perceived itch, discomfort/pain, and unusual visual sensations (e.g., phosphenes) during the vigilance task (see Appendix B for VAS questions).

tACS

tACS was administered through a battery driven constant current stimulator (DC Stimulator Plus, NeuroConn, Ilmenau/Germany) controlled through Spike2 software via a Power1401 mkII microcomputer (both Cambridge Electronic Design, Cambridge/UK). 5 x 7 cm^2 rubber electrodes in saline-soaked sponges (0.9% NaCl) with a thin layer of Sigma electrode gel were attached to the scalp with rubber bands and additionally supported by tubular surgical bandages to maximise the contact between electrode and scalp. tACS-electrodes were placed bilaterally over PO7/PO9 and PO8/PO10 of the 10/10-system (Figure 2.1 top; cf. Zaehle et al., 2010).

Individual stimulation frequency (ISF) and intensity were determined once, in the first session, for all four sessions. ISF was determined from resting EEG with eyes open. First, the Fast Fourier Transform (FFT) of the entire 2 min

recording was calculated (frequency resolution 0.5 Hz). The resulting spectrum at electrode POz was then used to identify the individual peak frequency in the α-range (8 - 12 Hz), which was chosen as the ISF. ISF ranged from 8 - 11 Hz across participants (see Table 2.1). The tACS intensity was adjusted below individual phosphene- and discomfort threshold using a staircase procedure. Eighty tACS cycles at ISF were administered with increasing intensity from 0.75 mA/peak-to-peak (pp) (at which all volunteers reported no or very weak sensations) in steps of 0.25 mA up to 2 mA/pp (maximum current density 0.02857 mA/cm^2 for a DC current, see Nitsche et al., 2003) or until the person reported phosphenes or perceived the stimulation as too uncomfortable. In this case intensity was decreased by 0.1 mA/pp until no phosphenes were detected and until the stimulation was acceptable to the participant. Intensities ranged from 1.35 - 2 mA/pp across participants (see Table 2.1).

tACS was administered in a within-subject design with three active conditions and one sham condition on four different days. A schematic of the protocols is shown in Figure 2.2. In all active conditions α-tACS at ISF was applied in an intermittent on/off pattern. Stimulation was programmed as a virtual sine wave of amplitude zero spanning the whole stimulation session, where amplitude was ramped up or set to zero during appropriate time intervals and at the appropriate phase angle (Spike2 software, Cambridge Electronic Design, Cambridge, UK). Total stimulation duration (amount of on-time) in each active condition was constant for any particular participant (7,200 α-cycles at ISF) but varied across participants due to the variability in individual posterior α-frequency (i.e., from approximately 11 min for an ISF of 11 Hz to 15 min for an ISF of 8 Hz). Total session duration was twice the length of total stimulation time (or equivalent for sham). Both duration and maximum current density are in line with current best practice and safety guidelines for DC applications in healthy participants.

Across conditions, we varied the length of single tACS-epochs (on-period) as well as phase-consistency across epochs as follows:

In the *short phase-continuous condition (ShortCo)* (Figure 2.2, first row), tACS was switched on for thirty cycles (i.e., on-periods of 3 s in participants with a 10 Hz-ISF) followed by an off-period of the same duration. This was repeated

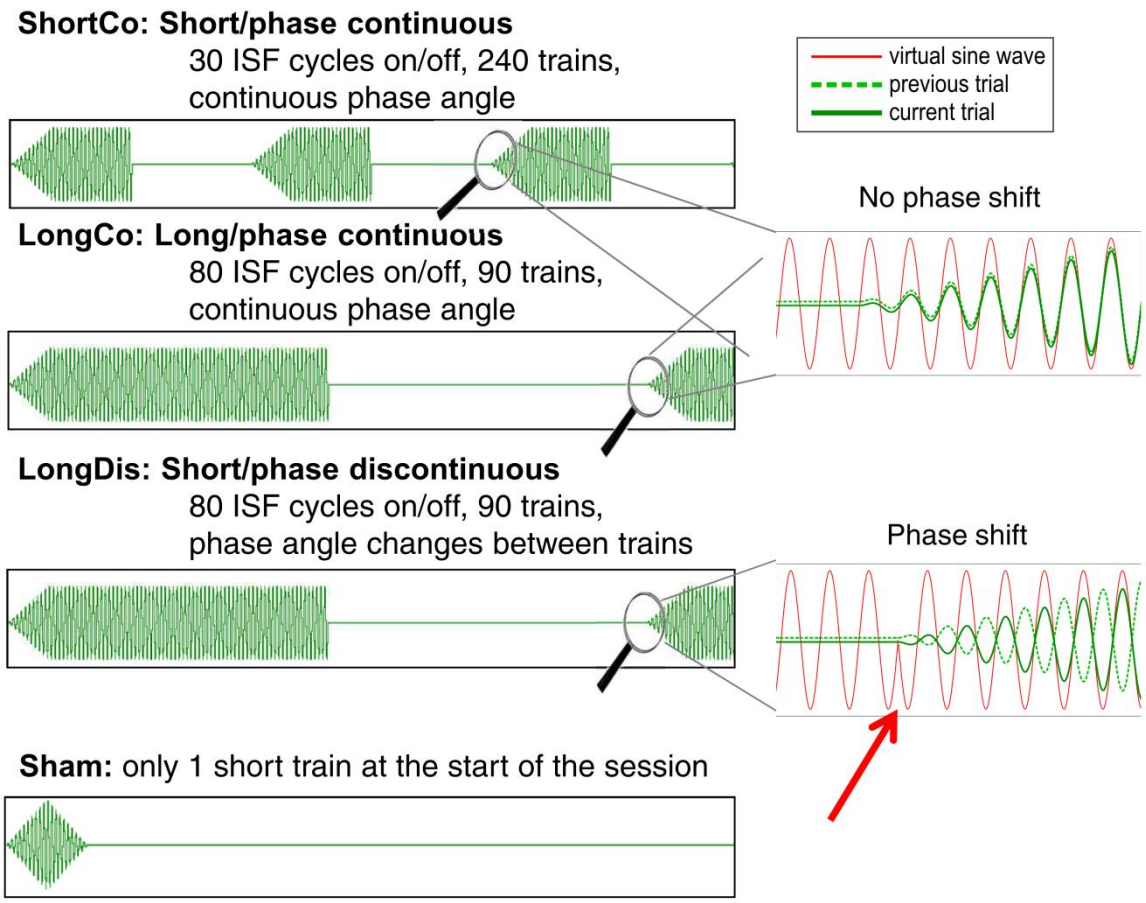

Figure 2.2: tACS protocols

Across conditions, we manipulated duration (ShortCo versus LongCo and LongDis) and phase continuity between successive tACS trains (ShortCo and LongCo versus LongDis). In this example, there is a 180° phase shift between trains in the LongDis condition (red arrow). For details refer to section tACS. ISF = Individual stimulation frequency.

240 times with phase continuity between successive on-states (i.e., by adjusting amplitude, but not phase, of a virtual sine-wave spanning the whole stimulation session). In the *long phase-continuous condition (LongCo)* (Figure 2.2, second row), tACS was switched on/off with phase continuity (as above) for eighty cycles (i.e., on/off for 8 s-epochs in participants with a 10 Hz-ISF) in ninety repetitions. The *long phase-discontinuous condition (LongDis)* (Figure 2.2, third row) was identical to LongCo, except that phase-continuity was disrupted across single tACS-epochs by introducing a phase shift of 0° , 90°, 180°, or 270° to the virtual sine wave during off-periods (approximately equal probability) with respect to the previous on-period, thus initiating tACS at a different phase angle. In all these conditions, tACS-intensity was ramped up to maximum intensity over the first ten cycles to minimise unpleasant sensations under the electrodes. Finally, in the *sham condition* (Figure 2.2, bottom row) only one short tACS-train (ten cycles ramp-up plus ten cycles ramp-down) was

administered at the beginning of the session to induce the skin sensations that are often experienced at the onset of electrical stimulation. This condition was included to control for tACS-unspecific effects (e.g., fatigue).

EEG recording

EEG was recorded at the midline sites Fpz, Fz, Cz, CPz, Pz, and POz (referenced to AFz with ground FCz according to the international 10/10 system) (Figure 2.1 top; cf. Zaehle et al., 2010) using a TMS/MRI compatible BrainAmp MRPlus amplifier (BrainProducts, Munich, Germany) and sintered Ag/AgCl electrodes. Vertical eye movements were recorded from two additional electrodes above and below the right eye. Electrode positions were determined manually, and the electrodes were attached with EC2 electrode paste and surgical tape. The signal was bandpass-filtered online between 0.1 - 1000 Hz and digitised at a sampling rate of 1 kHz (during the first recordings) or 5 kHz (in later sessions).

Visual change detection task

The task was programmed in Presentation software (version 16.3, Neurobehavioral Systems, Albany, US) and presented on a 20" CRT monitor (screen size 40 x 32 cm, resolution 1280 x 1024 pixels) at a viewing distance of approximately 1 m. Volunteers were asked to maintain fixation on a white cross centrally presented on a grey background (RGB values 131, 131, 131). A dark red disk (RGB 171, 69, 69, diameter 30 pixels or 0.5° visual angle) roughly isoluminant with the background was continuously presented in the lower central visual field (250 pixels or 4.5° below the centre point). Participants had to respond by mouse click as quickly as possible whenever the colour of the disk changed from red to green (RGB 69, 171, 69; duration 150 ms). If no response was registered within 1.1 s after target offset negative feedback was given by changing the colour of the fixation cross transiently from white to red. Target events occurred at low frequency after intervals of between 2.5 - 4.5 min duration and had low saliency to minimise interference of visual processing with induced alpha activity. Colour changes were also temporally uncorrelated with tACS on/off-periods. The number of trials for individual participants (5 – 7 trials) and their duration was calculated based on their respective ISF to maintain

constant visual event frequency and to match the duration of the task to that of the tACS protocol. Two breaks of 45 s were inserted after approximately one and two thirds of the task to allow participants to move and blink. While the stimulation protocol was continued during this pause, these trials, and trials containing a target event, were not included in the analysis. Due to the very low number of trials, the behavioural results were not further analysed.

EEG analysis

EEG preprocessing of the resting EEGs was done in BrainVision Analyzer 2.0 (BrainProducts, Munich, Germany). All other analyses were performed in MATLAB (MathWorks, Natick, USA) using the Fieldtrip Toolbox (Donders Centre for Cognitive Neuroimaging, Nijmegen, Netherlands). The reported results refer to the signal recorded at electrode POz except for one subject (03), where due to excessive noise in one condition Pz was chosen instead for all conditions.

Analysis of aftereffects in α-power (pre versus post-test)

The analysis of the pre- and post-tACS EEG measurements largely followed the method of (Neuling et al., 2013; Neuling, Rach, et al., 2012; Zaehle et al., 2010). The "eyes open" and "eyes closed" resting EEGs were segmented into one second epochs. Epochs containing eye movement and muscle contraction artefacts were discarded after visual inspection. A fast Fourier transform (FFT) for frequencies between 1 and 20 Hz (0.5 Hz resolution) was calculated for individual epochs using a Hanning window and 2 s zero-padding. The resulting spectra of each condition were averaged across epochs as well as across the individually determined α-bands (ISF ± 2Hz) per tACS-condition. Normalised relative changes of mean α-power from pre-test to post-test were calculated in decibel:

$$Change = 10 * \log10(post\text{-}test / pre\text{-}test)$$

Analysis of frequency-specificity of aftereffects

In order to assess whether tACS affected any other lasting broadband spectral changes or whether the effects are frequency-specific, the mean power spectra were subdivided into four non-overlapping canonical frequency bands

and averaged across the respective frequencies: delta/theta (1 - 3 Hz), theta (4 - 7 Hz), alpha (8 - 12 Hz), and beta (13 - 30 Hz; see Buzsáki, 2006; Helfrich, Schneider, et al., 2014). In addition, entrainment effects can be observed at subharmonics and harmonics of the tACS frequency (Ali et al., 2013). To evaluate this, power was averaged across frequency bands of 4 Hz width centered on 0.5, 2, and 3 times the individual stimulation frequency (first subharmonic, first and second harmonic, respectively). Change was calculated as for the ISF band described above.

Analysis of offline changes in α-activity in the intermittent, tACS-free intervals

Pre-processing. Due to rounding error in the calculations of the tACS sine wave, EEG triggers were offset over time, resulting in a phase shift of up to 20 milliseconds from the first to the last trial. Therefore, individual trials (epoched from 1 s before tACS onset until twice the duration of the tACS train and baseline corrected for the 1 s window preceding the artefact) were realigned by first finding peaks in the tACS artefact and by shifting these peaks to a common time line in order to assure that possible phase locking would not be diluted. Epochs of 2.3 s duration were extracted from the corrected EEG between successive tACS-trains, starting 100 ms after tACS-offset (due to residual tACS-artefact in the first 100 ms of EEG). Very noisy epochs and epochs with eye blinks at trial-onset were removed after visual inspection of the data. Remaining eye blink contaminations were then eliminated (1) using a principal component denoising approach (implemented in Fieldtrip) with the bipolar EOG-derivation as reference signal (using 1 - 8 Hz bandpass-filtered data to optimize blink detection, and applying the respective PCA-weights to the original data), and (2) by discarding the epoch if elimination was not successful. Because both long conditions had significantly lower trial numbers than Sham and ShortCo, as many trials as were available in the condition with the lowest trial number per participant were randomly sampled (without replacement) from all trials in each condition. All subsequent analyses were conducted on these subsamples of equal size.

Analysis of relative change in induced α-power. We followed a similar pipeline as for the analysis of the pre- and post-tACS data. From the pre-

processed data, two 1 s-epochs were cut at the beginning of each 2.3 s-interval. These were divided into blocks of early and late epochs, respectively (that is, first and second half of the experimental session). FFT-spectra were calculated for each 1 s-epoch separately, and subsequently averaged per block and tACS-condition. Average power in the individual stimulation band (ISF ± 2 Hz) for each block was again log-normalised to pre-test power.

Analysis of α-phase locking. To obtain phase information, pre-processed data were bandpass-filtered in individual α-bands (ISF ± 2 Hz) and Hilbert-transformed. The resulting complex values were normalised to unit amplitude. The phase locking value (PLV) was computed for each time point as the absolute value of the mean of these normalised complex values across trials. PLVs were averaged across the first 200 ms of the 2.3 s-epoch (i.e., from 100 – 300 ms post artefact) and then across epochs within early and late blocks in each tACS-condition.

Analysis of online changes in α-activity during tACS-on intervals

Online tACS artefact removal was attempted by subtracting a scaled and shifted sinusoid at ISF (subjects 01 - 06) or the independently recorded channel containing the stimulation artefact (subjects 07 - 12) to obtain estimates of alpha power changes during tACS. However, these attempts were not successful in removing the tACS-induced noise satisfactorily, and in some cases even added harmonic artefacts. Therefore, these analyses are not further described here.

Statistics

All statistical comparisons were computed in IBM SPSS Statistics, version 19.0, IBM Corp, Armonk/US). Nonparametric statistical tests were used for the following reasons. First, verifying the assumption of normality (for instance by using Kolmogorov-Smirnov or Shapiro-Wilk's W tests for normality) in a small sample is unreliable because of low power. Although the alpha power change data seemed fairly normally distributed, there were two outliers in the LongDis condition and one in the sham condition (criterion: greater or smaller than 1.5 times the interquartile range), two of which remained outliers when ignoring stimulation condition and which moreover belonged to three different

participants (see also Figure 2.5, left). Second, the decibel scale at which changes in alpha power were quantified is inherently non-linear, which violates the assumption of equal intervals that is required in analysis of variance (ANOVA) (Lowry, 1998; Stevens, 1946). Fundamentally, there is no a priori reason to believe in simple linear behaviour of alpha activity. To this adds a lack of published data that might allow an educated guess about the population distribution of tACS-induced alpha changes. We therefore used the non-parametric Friedman test to analyse our data, which does not require that distributions meet stringent criteria and only requires that data can be meaningfully ranked. This test can be considered an alternative to a one factor repeated measures ANOVA on ranks, and only assumes that the measurements are independent between participants and are at least on an ordinal scale (Lowry, 1998). The null hypothesis is that all protocols are equally effective (or ineffective) in producing alpha changes. The alternative hypothesis is that at least one protocol consistently produces larger (or smaller) changes than other protocols. To follow up significant results on the Friedman test, Wilcoxon signed rank tests (using the normal approximation method) were employed. This non-parametric alternative to the paired samples t-test uses ranks instead of raw score differences but does take the magnitude of the differences into account, and can be more powerful than the t-test if the assumptions for the latter do not hold (Howell, 2007). It relaxes the requirement for normality of differences and only assumes symmetry. The null hypothesis is that the median difference between two protocols is zero.

For the analysis of the relationship of the mismatch between endogenous and stimulation frequency with the magnitude of α-enhancement relative to sham, the non-parametric Spearman's rank order correlation was used. Unlike Pearson's product-moment correlation, this test relaxes the assumption of linearity and only requires that the response variable is monotonously associated with the predictor.

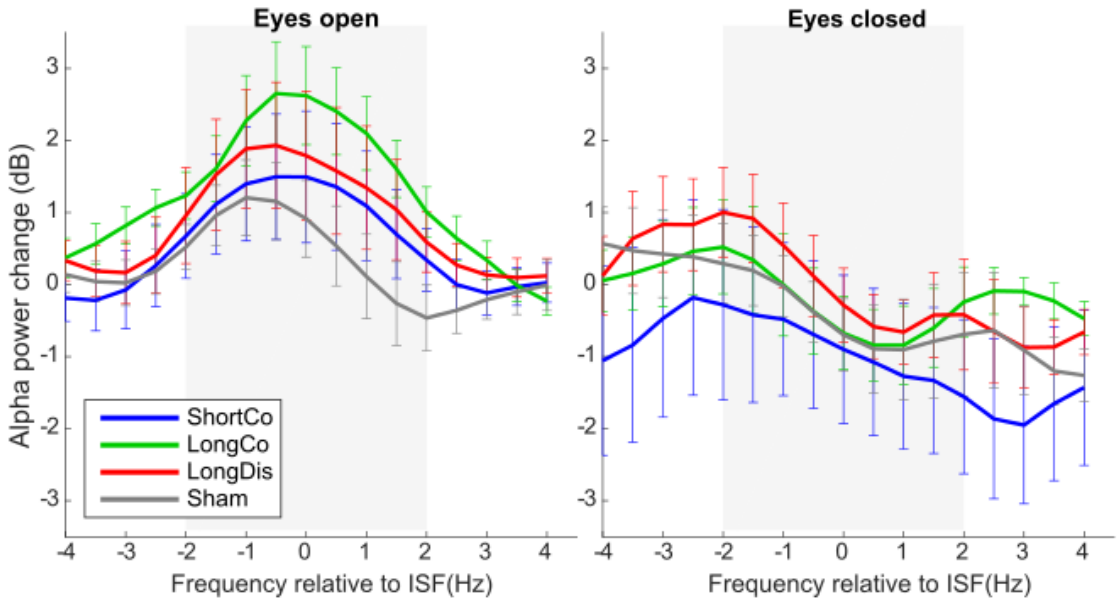

Figure 2.3: Change in alpha power spectra at rest with eyes open and closed

Grand mean change in resting EEG power in the individual α-band from pre-test to post-test with eyes open (left) and eyes closed (right) in dB. Spectra were aligned to individual stimulation frequency (ISF) before averaging across participants. Grey shaded areas shows frequency range over which spectral change was averaged for statistical analysis (ISF ± 2 Hz). Error bars represent standard error of the mean (*N* = 12).

Results

α-Aftereffect replicated with intermittent α-tACS when eyes are open

α-power (ISF ± 2 Hz) at rest with eyes open was enhanced after intermittent α-tACS (pre versus post-test), with participants showing on average stronger α-enhancement after active tACS as compared to sham (see Figure 2.3, left panel for the grand mean change in the power spectrum, Figure 2.4A for grand mean change in α-band power). Specifically, in both long conditions individual responses were highly consistent across participants, with 11 out of 12 participants showing stronger α-enhancement to α-tACS in the long phase-continuous and 10 out of 12 in the long phase-discontinuous condition as compared to sham (Figure 2.4B, middle and right panel: LongCo versus Sham and LongDis versus Sham). Figure 2.5 shows the distribution of the group data (left) and individual results per participant (right).

Statistically, a main effect of condition was confirmed by a Friedman Test ($X^2(3) = 11.1$, $p = .011$). Breaking down this effect using the Wilcoxon Signed

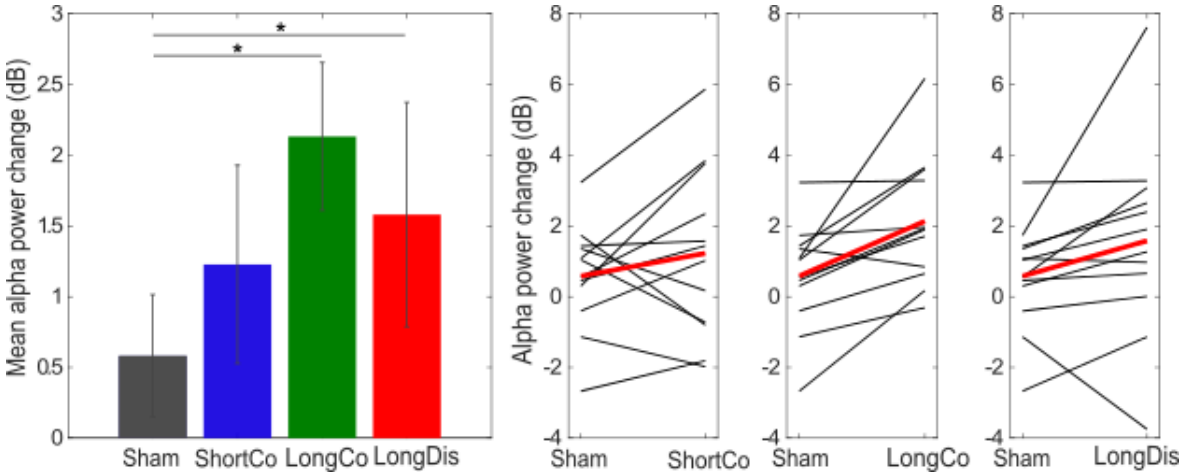

Figure 2.4: Change in alpha band power at rest with eyes open.

Left: Grand mean change in resting EEG alpha power (in dB) as in Figure 2.3 (left panel) averaged across frequencies within the individual α-band (individual stimulation frequency ISF ± 2 Hz). Spectra were aligned to ISF before averaging across frequencies and participants. Black horizontal lines with asterisks indicate the conditions that are statistically different at alpha = .05. Only the respective comparisons between Sham and LongCo (lower brace), and Sham and LongDis (upper brace), were significant. Error bars represent standard error of the mean (N = 12). *Right:* Differences in α-power change between sham and each active tACS condition per participant. Black lines represent individual differences; red lines represent group mean difference. Most volunteers show a greater increase after stimulation with long (80 cycles at ISF) trains compared to Sham.

Rank Tests (2-tailed) indeed revealed significant α-enhancement only for both long tACS conditions compared to sham (LongCo versus Sham: $Z = 2.82$, $p = .005$; LongDis versus Sham: $Z = 2.04$, $p = .041$; ShortCo versus Sham: $Z = 1.26$, p = .21), replicating the α-aftereffect previously reported for continuous α-tACS-protocols in eyes open conditions (Helfrich, Schneider, et al., 2014; Neuling et al., 2013; Neuling, Rach, et al., 2012; Zaehle et al., 2010). In accordance, only after LongCo and LongDis tACS, the change from pre- to post-test was significantly greater than zero (Wilcoxon One Sample Signed Rank Test, LongCo: $p < .001$, LongDis: $p = .032$; all other $p = .075$, one-sided, uncorrected). Only for LongCo tACS, this result survived Bonferroni correction.

Alpha aftereffect does not differ between phase-continuous and phase-discontinuous protocols

Alpha enhancement after active tACS (LongCo > LongDis > ShortCo) did not significantly differ between conditions (all $p > .05$). While long intermittent tACS significantly enhanced α-power (relative to sham), this enhancement was observed irrespective of phase-continuity between tACS-trains. Hence,

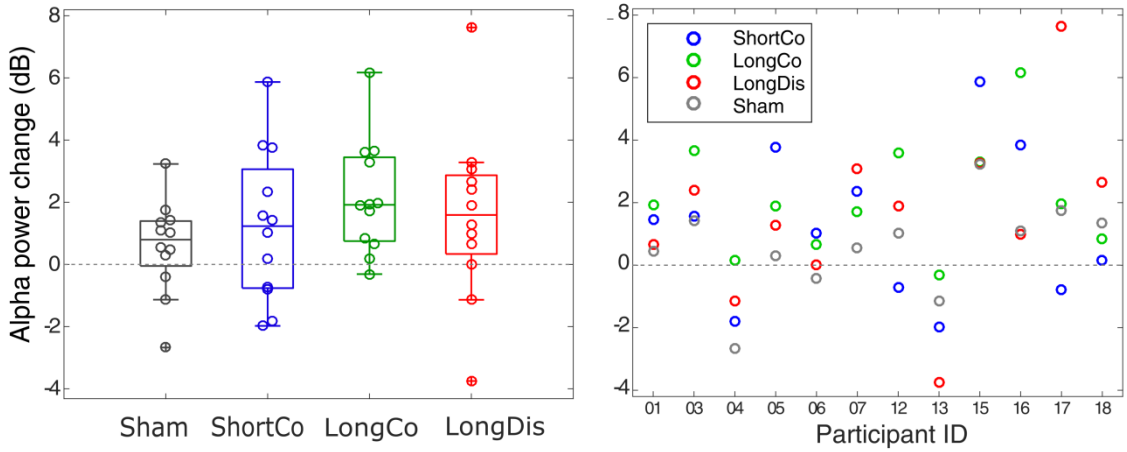

Figure 2.5: Alpha power change in individual participants (eyes open)

Circles represent the change for individual participants from pre- to post-test within each protocol at rest with eyes open. *Left*: Change distribution per protocol. Boxes show 25/50/75th percentile, whiskers enclose 1.5 * interquartile range. *Right*: Same data as left but now rotated and grouped per subject. Grey dotted lines in both plots represent no change.

introducing phase jitter during tACS did not disrupt the α-aftereffect, which speaks against prolonged entrainment echoes contributing to the aftereffects.

Alpha aftereffects do not peak at stimulation frequency, but at preferred cortical frequency

While we stimulated at a fixed frequency (individual stimulation frequency/ISF = individual α-frequency/IAF on day 1), several participants showed variable IAF across sessions. This was established by randomly sampling (1000 repetitions with replacement) and averaging subsets of spectra from 1 s epochs in pre-test-EEG within each session to extract peak frequency in the 8 - 12 Hz-range. IAF on a given day was defined as the mode of these peaks. As a consequence, ISF deviated from IAF between sessions for several participants (ISF minus IAF: range from -1.5 Hz to +3.0 Hz, see also Figure 2.6A). This allowed us to assess whether aftereffects peaked at ISF or spontaneous IAF. Note that ISF was in most cases slightly below the IAF of a given session.

Breaking down the α-band into nine bins (IAF - 2 to IAF + 2, in 0.5 Hz steps) (Figure 2.6B), we found that tACS-aftereffects (LongCo > LongDis > ShortCo) peaked at IAF and IAF +0.5 Hz (rather than ISF). In other words, they did not show the left-skew of the ISF histogram (Figure 2.6A). Separate Friedman Tests

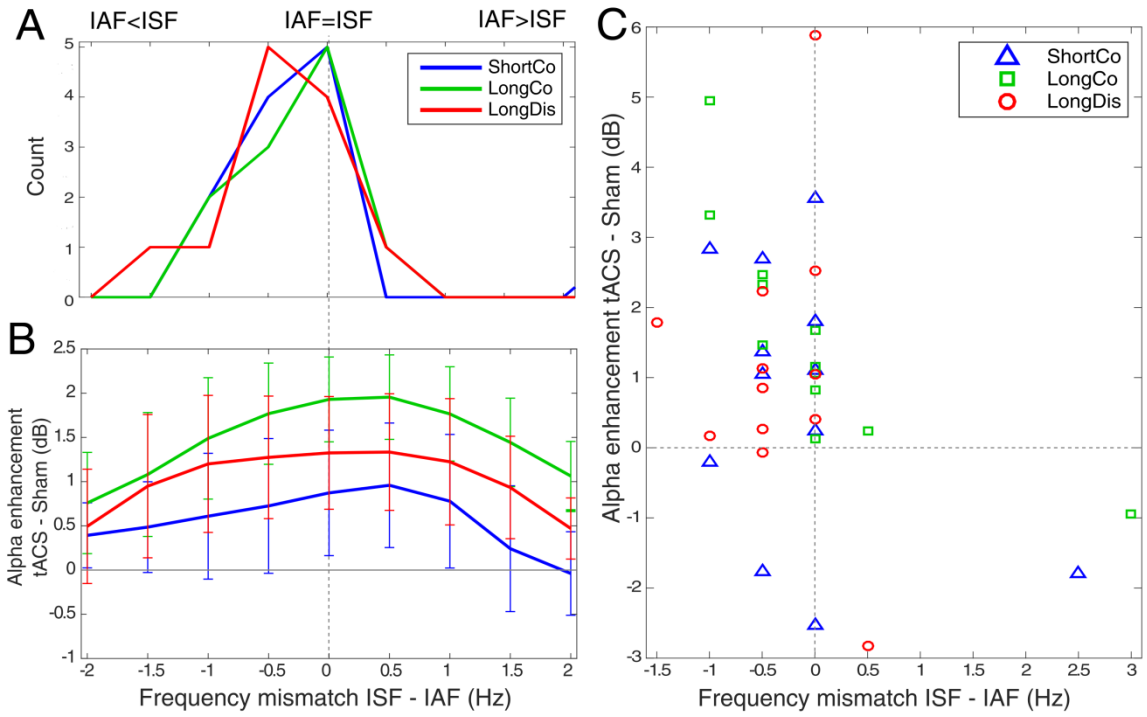

Figure 2.6: Dependence on frequency mismatch

A) Individual stimulation frequency (ISF) relative to IAF. The distribution shows that there was a tendency to stimulate at a lower frequency than the "optimal" alpha frequency. *B)* IAF-aligned alpha aftereffects (difference between active protocols and sham) in mean relative power increase from pre-test to post-test (dB). Frequencies within the individual alpha band are defined by the individual alpha frequency (IAF) measured on the day of each session. The average increase tended to be stronger at IAF and above, i.e., slightly higher than at ISF. Error bars represent standard error of the mean (N = 12). *C)* Correlations between relative alpha increase and extent of the mismatch between ISF and IAF. Data points to the left of the origin show sessions during which stimulation frequency was lower than the actual peak (established before each session). At least for the most effective protocol (LongCo), greater mismatch is associated with stronger alpha increase.

on the relative α-increase in the IAF-centred α-band and the two flanker α-bands (IAF – 2 Hz to IAF - 1 Hz/ IAF + 1 Hz to IAF + 2 Hz) revealed significant aftereffects in the IAF-centred band ($X^2(3) = 8.1$, $p = .044$) and the higher α-band ($X^2(3) = 9.0$, $p = .029$). At the IAF-centred band, the contrasts of both LongCo- and LongDis-conditions against Sham were significant (Wilcoxon Signed Rank Test; LongCo: $Z = 2.90$, $p = .004$; LongDis: $Z = 1.96$, $p = .05$; all other $p > .05$). In the higher α-band, only the contrast of LongCo versus Sham was still significant ($Z = 2.51$, $p = .012$; LongDis versus Sham: $Z = 1.73$, $p = .08$). In addition, after LongCo there was also greater increase compared to ShortCo ($Z = 2.51$, $p = .012$). Importantly, repeating the same analysis but now centred on ISF (instead of IAF) did not reveal significant tACS-related α-aftereffects at ISF (ISF - 0.5 Hz to ISF + 0.5 Hz, Friedman $p > .05$). Hence, tACS-induced aftereffects were observed at or above the preferred cortical frequency but not

at stimulation frequency, which again is inconsistent with prolonged entrainment echoes contributing to the aftereffect.

No enhancement of α-aftereffects when stimulation and preferred frequency match

It could be argued that the previous result can be explained by entrainment if the magnitude of aftereffects followed the pattern of an Arnold Tongue, with those participants whose stimulation frequency matched the intrinsic frequency contributing most to the aftereffects, relative to those participants with mismatching frequencies. To address this argument directly, we took advantage of the variability of IAF relative to ISF. To assess the dependence of α-enhancement on the ISF-to-IAF match in any given session, we calculated the correlation between α-enhancement (defined as alpha power change in active tACS minus alpha power change in Sham) and stimulation frequency mismatch (= ISF minus IAF). We found that no active tACS-condition showed stronger α-enhancement with better match between ISF and IAF (Figure 2.6C). Instead, we found a significant inverse relationship in the most effective condition (LongCo), with stronger tACS-induced α-enhancement for greater deviations between ISF and IAF (Figure 2.6C, green rectangles, Spearman's *rho* = -.93, $p < .001$, corrected for ties). This association remained strong even with the two most extreme cases removed (Spearman's *rho* = -.83, $p = .003$). A correlation derived from a small sample must be considered with caution but the data show that α-enhancement does not depend on a perfect match between ISF and IAF, contrary to what would be expected from entrainment echoes, and in favour of plasticity effects.

Frequency-specificity: No aftereffects in other frequency bands or at (sub)harmonics

Regarding unspecific spectral aftereffects, mean changes in power from pre-test to post-test appeared to be largest in the alpha band, particularly in the active conditions (Figure 2.7A). Outside the alpha band, the power changes at other frequencies did not obviously differ across tACS conditions, with a possible exception of a delta power decrease in the LongDis condition which, however, failed the significance test. Separate Friedman tests with factor tACS

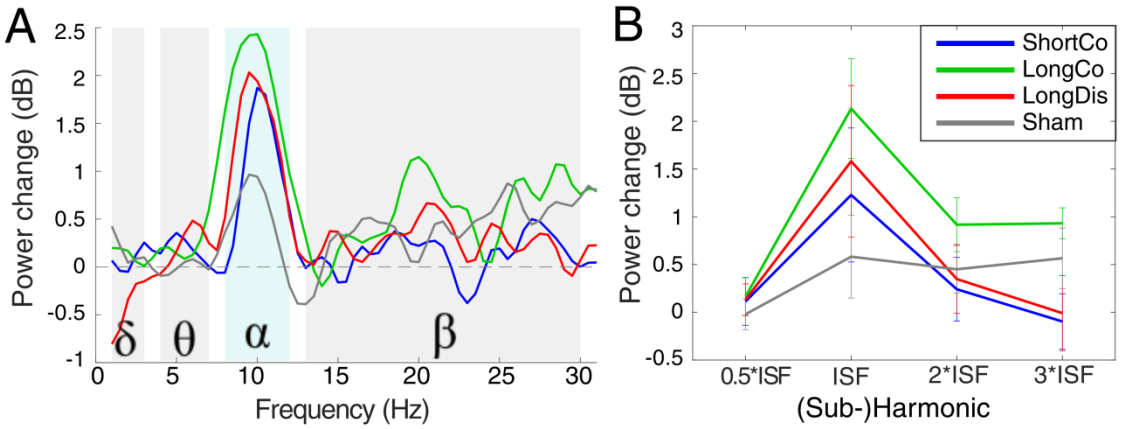

Figure 2.7: Frequency-specificity of power enhancement

A) Mean power change in canonical frequency bands (*N* = 12). Only the alpha band (blue shaded area) showed a significant increase in power following both long tACS conditions compared to Sham. Grey shaded areas indicate the borders of each frequency band. *B)* Mean power change at first subharmonic and first and second harmonic of individual ISF. Error bars represent standard error of the mean.

condition were calculated for each band separately (note that only one factor can be tested at a time). Only in the alpha band the test was significant ($X^2(3) = 11.1$, $p = .011$), confirming the corresponding effect for the same data centered at ISF. This result also survives Bonferroni correction to account for the multiple separate tests across frequency bands ($p = .044$). The test results for the other bands were: delta, $X^2(3) = 2.0$, $p = .57$; theta, $X^2(3) = .9$, $p = .83$; beta, $X^2(3) = 6.0$, $p = .11$ (all uncorrected). Post hoc Wilcoxon signed rank tests repeated the pattern seen for the ISF band, with LongCo and LongDis being significantly different from Sham (LongCo versus Sham: $Z = 2.82$, $p = .005$, LongDis versus Sham: $Z = 2.12$, $p = .034$) but not from each other (LongCo versus LongDis: $p = .35$; all other $p > .13$).

For the first subharmonic ($X^2(3) = 1.2$, $p = .75$), and the first ($X^2(3) = 4.2$, $p = .24$) and second harmonic ($X^2(3) = 6.0$, $p = .11$; all uncorrected), there were no significant differences between protocols (Figure 2.7B). These results suggest that the effect of tACS is highly specific.

No effect of tACS on alpha power with eyes closed

While α-aftereffects of α-tACS were observed in the eyes open resting state, no aftereffects were observed in the eyes closed state (compare Figure 2.3 left versus right panel). An analysis of the resting data with eyes closed averaged across ISF (identical to the analysis of the eyes open resting

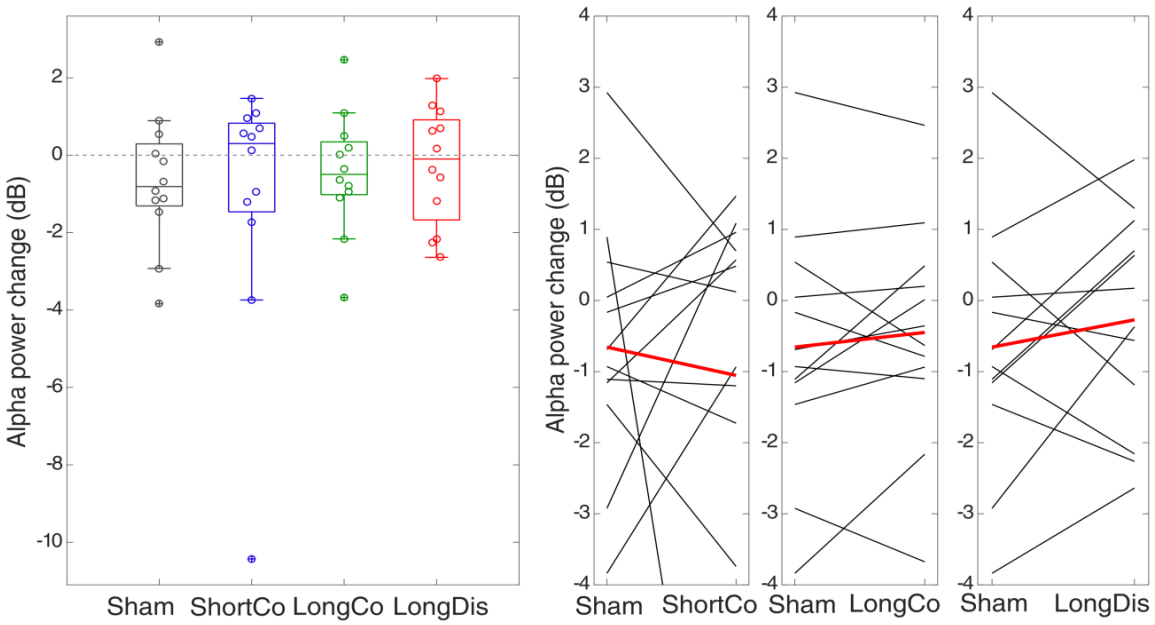

Figure 2.8: Alpha power change in individual participants (eyes closed)

Circles represent the change for individual participants from pre- to post-test within each protocol at rest with eyes closed. *Left*: Change distribution per protocol. Boxes show 25/50/75th percentile, whiskers enclose 1.5 * interquartile range. *Right*: Differences in α-power change between sham and each active tACS condition per participant. Black lines represent individual differences; red lines represent group mean difference. There was no group effect of tACS protocol.

state data) yielded no significant effect of tACS condition (Friedman test, $X^2(3) = .4$, $p = .94$; Figure 2.8; compare to Figure 2.5 for eyes open data). In addition, in no case was the change different from zero (Wilcoxon One Sample Signed Rank Test; all $p > .15$).

No lasting phase locking in intermittent, tACS-free intervals

The pattern of tACS-induced α-power changes in the intermittent intervals during stimulation (Figure 2.9, top row) was suggestive of a progressive build-up of the α-aftereffects. However the effect of tACS was not significant in either the early or late block (early/late: $X^2(3) = 6.0$ / 4.7, $p = .112$ / $.195$). Critically, we found no evidence of increased phase-locking (versus sham) in these intervals (i.e., after around eight seconds of stimulation with individual tACS trains; Figure 2.9, bottom row) (early: $X^2(3) = 2.5$, $p = .48$; late $X^2(3) = .7$, $p = .87$), again disagreeing with entrainment echoes contributing to the tACS-aftereffects. The absence of phase-locking immediately after tACS offset shows that online entrainment (if present) does not outlast the tACS trains even between individual trials, and rules out the survival of entrainment echoes for several minutes.

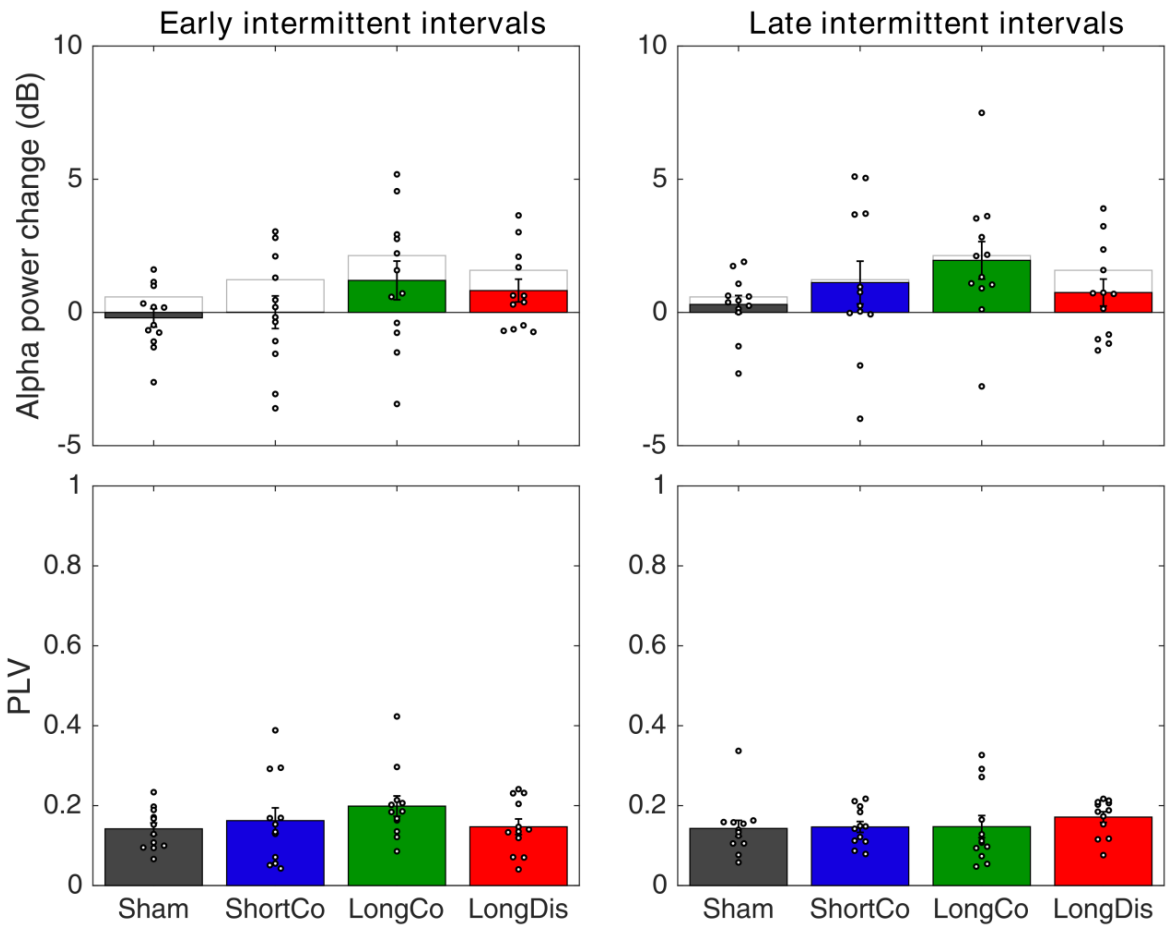

Figure 2.9: Alpha-effects in intermittent, tACS-free intervals

Top row: Relative increase (dB) in individual α-band power for early (left) and late (right) trials during tACS-free periods between stimulation trains compared to pre-test. Grey outline shows mean increase between pre-and post-test for each condition (as shown in Figure 4, left). *Bottom row*: Phase locking value (PLV) across trials for early (left) and late (right) trials. A value of 0 means no phase locking; a value of 1 means perfect phase locking. There is no evidence for enhanced phase locking. Bar charts show within-group mean, error bars represent the standard error of the mean (*N* = 12). Black circles are individual data points.

Peripheral effects of tACS (VAS scores)

The strength of sensations associated with tACS (Itch, Discomfort, and Visual) was rated on a continuous visual analogue scale from zero to ten, where zero means no sensation at all and ten means strong sensations. The resulting VAS scores are summarised in Table 2.2 and shown in Figure 2.10. Generally, mean scores were low for all three measures with right-skewed distributions, although higher ratings for Discomfort or Visual sensations were typically given after active tACS rather than sham (Figure 2.10A). The observation that only some participants felt the stimulation at all, while others claimed to be oblivious, is reflected in the somewhat bimodal distributions (e.g., Discomfort LongCo, Visual LongDis). Only for the Discomfort rating, this resulted in a significant group

Table 2.2: VAS scores of peripheral sensation ratings

VAS scores are reported per condition as median (interquartile range), *N* = 12.

VAS score	*ShortCo*	*LongCo*	*LongDis*	*Sham*
Itch	1.15 (1.8)	0.35 (2.41)	0.50 (2.09)	0.25 (1.40)
Discomfort	0.35 (0.83)	0.20 (2.25)	0.13 (0.26)	0.10 (0.16)
Visual	0.10 (0.08)	0.10 (0.20)	0.10 (0.94)	0.10 (0.11)

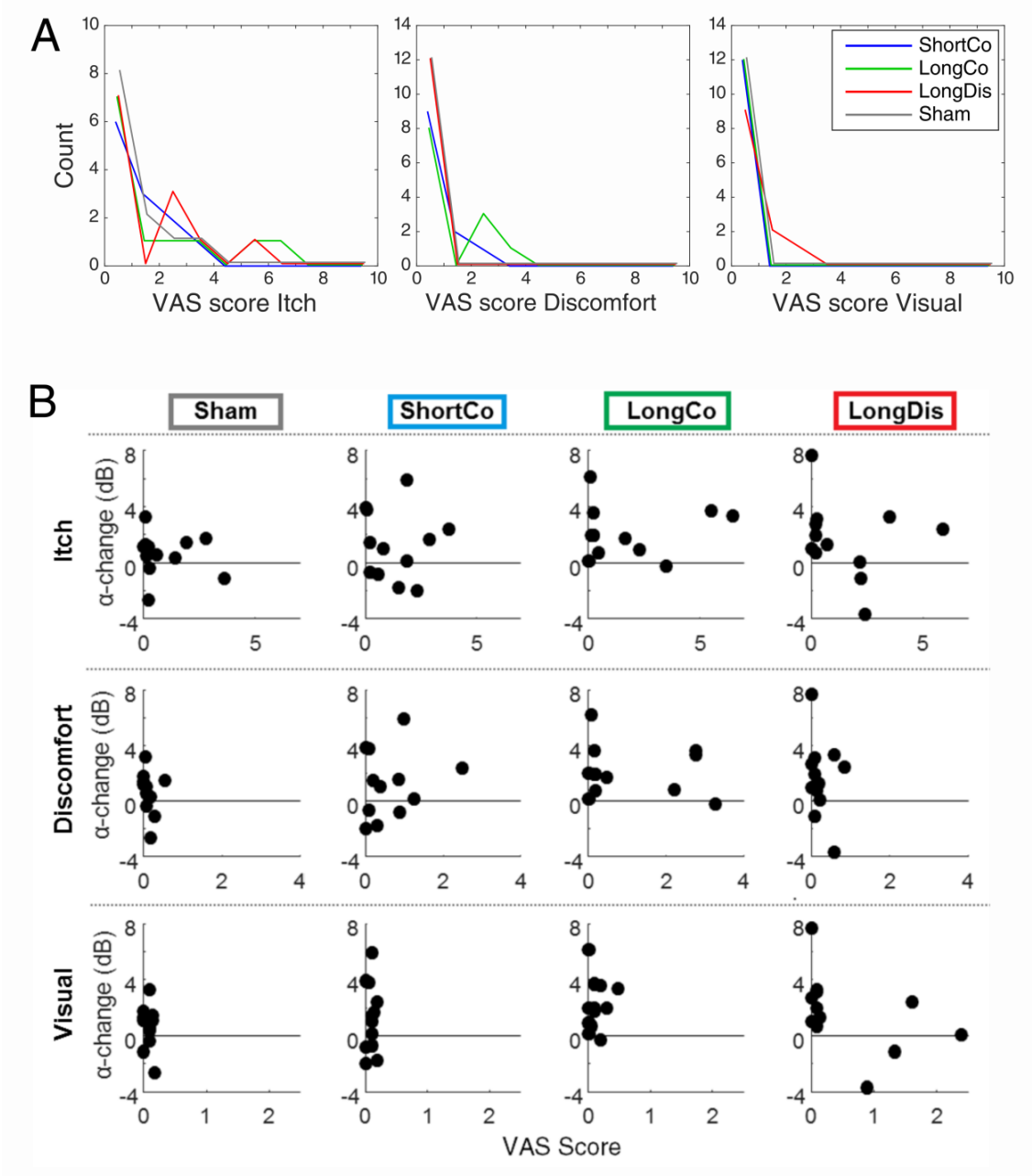

Figure 2.10: VAS scores for peripheral sensations

A) Histograms show similar distributions of VAS scores for ratings of itchiness (Itch), discomfort/pain (Discomfort), and unusual visual sensations (Visual) for active tACS and sham. *B)* Scatterplots show the (lack of) association of alpha power change with the intensity of peripheral sensations.

difference (Friedman's test, $X^2(3) = 10.0$, $p = .019$, uncorrected). A follow up Wilcoxon signed rank test for Discomfort ratings was performed on square root-transformed data to alleviate skewness and thereby satisfy one of the test's assumptions that the pairwise differences should be symmetrically distributed around their median. Except for the difference between both phase continuous conditions (ShortCo versus LongCo: $p = .68$), all other contrasts were significant or "approached" significance (ShortCo versus LongDis: $p = .098$; ShortCo versus Sham: $p = .053$; LongCo versus LongDis: $p = .031$; LongCo versus Sham: $p = .025$; LongDis versus Sham: $p = .094$), although none of these results survived adjusted Bonferroni correction. Qualitatively, where available, participants reported that they felt sensations they attributed to tACS either only very early in the experiment or only occasionally but not constantly. Overall, therefore, it is difficult to judge whether peripheral sensations had a causal influence on the observed differences in offline alpha power changes. It is highly plausible that sensations differ between sham and active conditions and should not be discounted from any interpretation of tACS effects. However, visual inspection of the association between VAS scores and alpha change provides no reason to believe this is the case in our sample (Figure 2.10B, see in particular the second row pertaining to Discomfort ratings).

Discussion

This experiment tested in a novel intermittent tACS paradigm to what extent α-aftereffects in response to α-tACS *i)* show characteristics of neural entrainment, and *ii)* depend on prolonged phase stabilisation. To this end, we manipulated phase continuity and train duration in three discontinuous tACS-protocols with constant total stimulation time and compared tACS-induced offline α-changes against sham.

Replication of previous observations

In brief, we replicated the previously reported finding of offline α-enhancement (Helfrich, Schneider, et al., 2014; Neuling et al., 2013; Neuling, Rach, et al., 2012; Zaehle et al., 2010) after active α-tACS using eighty cycle on-off protocols. This enhancement was exclusive to the eyes open condition, in line with earlier observations of state-dependent tACS effects that are abolished

by ceiling levels of alpha power at baseline when eyes are closed (Neuling et al., 2013). As in previous reports, no spectral changes in other frequency bands were present, suggesting frequency-specificity of stimulation effects. However, the absence of a tACS-frequency harmonic effect with a peak in the beta range may also simply reflect the lower signal to noise ratio at higher frequencies and needs to be interpreted with caution.

Entrainment or plasticity?

No dependence on phase continuity

The novel contribution of this experiment was the examination of α-aftereffects for characteristics of entrainment. We found that phase discontinuity did not disrupt the build-up of an aftereffect. Given that previous demonstrations of entrained oscillations were short-lived and subsided within a few cycles (Hanslmayr, Matuschek, & Fellner, 2014; Marshall et al., 2006; Thut, Veniero, et al., 2011), it could be argued that summation of phase entrainment over many trials might not be feasible in principle; in other words, that it is unlikely that the effects of any single tACS train should last long enough to allow the sort of interaction between successive trains that would be required for the amplification or disruption of a phase effect such as hypothesised in this experiment. Indeed, there was no evidence of enhanced phase-locking (measured by PLV) across the duration of silent intervals relative to tACS offset in any active condition, indicating that entrained activity after each tACS train was either absent or too transient to be detectable. In our hypothetical scenario, the wave patterns of phase-continuous trains (with identical starting phase angles), if superimposed, align perfectly onto one sinusoid. If alpha activity remained stably phase-locked to the tACS current waveform even in the absence of stimulation, one would therefore expect to see a good overlap of EEG traces between trials. This would be reflected in the clustering around certain phase values, and therefore a high PLV, at each time point. On the contrary, the wave patterns of phase-discontinuous trains, if superimposed, produce four distinct sinusoids (relative to each of the four starting phase angles) which cancel one another out if averaged. Therefore, the phase angles at each time point would cancel out, and the PLV would be low. In our data set, the relative PLVs between all three active conditions and sham were, however, statistically

indiscernible. As there were substantial offset artefacts, no conclusions can be made about the short interval immediately following tACS. Regardless, any hypothetical phase-alignment subsided before the analysed segment, that is, within less than 150 ms.

Along the argument of insufficient longevity, our phase manipulation would be ineffective in principle as it would target oscillatory activity in an inappropriate time window, long after such an interaction might have an impact. However, along the same argument it is implausible that single tACS events produce entrainment that remains detectable over an average of up to two minutes. Independent of these considerations, we found alpha enhancement which needs to be accounted for, regardless of whether our manipulation was ineffective by design.

Alpha enhancement irrespective of frequency mismatch

We did not observe the expected greatest enhancement after sessions in which the stimulation frequency (ISF) and the day-to-day alpha peak frequency (IAF) were perfectly matched. Moreover, power enhancement relative to sham was greatest at or above the day-to-day alpha peak frequency, rather than at stimulation frequency, which tended to be lower than the IAF. While this might be explained by a non-linear effect with lesser enhancement for mismatching frequencies, as might be expected in the presence of an Arnold Tongue, we also show that the magnitude of frequency mismatch was not inversely (indeed sometimes even directly) associated with alpha enhancement. In other words, in a number of sessions during which there was a relatively large mismatch between tACS frequency and intrinsic alpha frequency we observed greater alpha enhancement than in other sessions with a better match but only small power changes. In the LongCo condition, this relationship was captured by a significant linear correlation. This is surprising and contrary to the prediction from entrainment that smaller mismatch should give rise to larger effects. Due to the small sample size and lack of agreement between the active conditions this statistical result begs for replication, ideally over a larger range of mismatching frequencies to assess the dynamics of this relationship.

While simulations and in vitro work have shown that frequency-matched tACS should produce the greatest response *during* stimulation (Ali et al., 2013) unambiguous evidence for such a correlation in vivo (neural or behavioural) still has to be supplied. Even less is known about the frequency-specificity of tACS aftereffects, although our results match those of Helfrich and co-workers, who found no relationship between IAF before stimulation and α-power increase after 10 Hz tACS (2014). Moreover, as reviewed in Veniero et al. (2015), neural changes in power or coherence after rhythmic electrical stimulation are often not exclusive to, or may not even be present at, the stimulation frequency.

All taken together, the presented results conflict with the hypothesis that the α-aftereffect in our intermittent protocol are simply entrainment echoes, and are more supportive of plasticity as the underlying cause.

Duration of stimulation may determine magnitude of aftereffects

Our results show in addition that only long (approx. 8 s) but not short (3 s) intermittent tACS trains could induce statistically significant alpha enhancement compared to sham on a group level. The cause for this difference in effectiveness cannot be derived from the current data set. One can speculate that effectiveness of intermittent tACS might depend either on the duration of the trains, the duration of the silent intervals, or the specific combination thereof. Assuming that the aftereffect is a function of online entrainment, longer trains may lead to stronger entrainment effects (i.e., a more stable oscillatory state) and possibly stronger ensuing plastic changes. Alternatively, longer intervals between successive electrical events may facilitate the consolidation of short-term network changes. The respective contribution of these factors could be tested by manipulating the relative tACS-to-interval duration. Of note, a lack of alpha changes after short tACS intervals has also been reported after stimulation with 300 1 s on-trains and silent intervals of 4 - 7 s (Strüber, Rach, Neuling, & Herrmann, 2015). In this study the length of the silent intervals was jittered and was on average intermediate between the durations used in the current design. The authors suggest that such short tACS episodes may not be sufficient to induce plasticity mechanisms, independent of whether they arise from entrainment or other processes. This has practical implications for the design of future tACS experiments as it suggests that one

may be able to manipulate oscillations online using short trains of three seconds (as in our protocol) or less without the confound of progressively stronger aftereffects. This is particularly relevant for event-related designs and protocols that suffer from order effects. It needs to be established, however, that stimulation at different intensities and frequencies produces equivalent aftereffects. As reviewed in the introductory chapter and in Veniero et al. (2015), it is far from obvious that this must be the case.

Two possible caveats should be pointed out in the discussion of this result. Firstly, because there were more trains for the short compared to the long protocols (240 and 90 trials, respectively), but equal tACS-on time, more time was spent during ramping in ShortCo versus LongCo (that is, 1/3 versus 1/8 of total stimulation time) and accordingly less time was spent during stimulation at full amplitude (2/3 versus 7/8 of total stimulation time). It is therefore possible that the results were more variable after ShortCo because overall less energy was delivered to the brain. Secondly, the lack of a group effect for short tACS is reflected on an individual level (Figure 2.5 right) by only 8/12 participants with a greater alpha increase relative to Sham, compared to 11/12 and 10/12 for the long conditions. In other words, the statistical test result hinges on the results of only two participants. Given that in three cases, ShortCo was the *most* effective protocol in terms of α-power changes, it is unclear whether this lack of change reflects lack of effectiveness or a general power problem due to this small sample size. In particular, to establish whether there might be a bimodal distribution of participants who responded to a protocol and those who did not, a much larger group needs to be examined.

Does tACS-induced plasticity depend on entrainment?

Despite growing evidence for entrainment *during* tACS (Feurra, Bianco, et al., 2011; Helfrich, Schneider, et al., 2014; Neuling, Rach, et al., 2012; Pogosyan et al., 2009; Strüber, Rach, Trautmann-Lengsfeld, Engel, & Herrmann, 2014; Voss et al., 2014), our findings indicate that online tACS-entrainment is not stable enough to outlast stimulation, while offline tACS plasticity effects can be present in the absence of phase stability. A similar distinction between online and offline effects has been made for transcranial magnetic stimulation (TMS): Short bursts of rhythmic TMS enhance brain oscillations at TMS-frequency during

(i.e., online to) TMS by immediate entrainment (Hanslmayr et al., 2014; Jaegle & Ro, 2014; Romei et al., 2016; Thut, Veniero, et al., 2011), but prolonged TMS leads to longer-lasting effects on brain oscillations that have been attributed to other mechanisms (i.e., long term potentiation or -depression) (Thut & Miniussi, 2009; Thut & Pascual-Leone, 2010; Veniero, Brignani, Thut, & Miniussi, 2011). An open question is to what extent online entrainment effects and offline plasticity effects are independent. Below we discuss, in light of our and related recent findings, two plasticity models, which assume independence versus dependence of online entrainment and offline plasticity effects, respectively.

Entrainment-independent plasticity: Pattern-invariant long-term potentiation or depression

Long-term plasticity and associated effects on brain oscillations have been observed without fine-tuning the stimulation frequency to specific neuronal circuits. For instance, prolonged transcranial direct current stimulation (tDCS), which has no oscillatory component and whose effects have been associated with changes in excitability and synaptic efficacy (Antal, Paulus, & Nitsche, 2011; Nitsche et al., 2008; Nitsche & Paulus, 2000; Rahman et al., 2013), may also lead to enhanced α-activity (Hsu, Tseng, Liang, Cheng, & Juan, 2014; Puanhvuan, Nojima, Wongsawat, & Iramina, 2013; Spitoni, Cimmino, Bozzacchi, Pizzamiglio, & Di Russo, 2013). Aftereffects of both TMS and tDCS have been related to long term depression (LTD) and potentiation (LTP) (Antal, Paulus, et al., 2011; Brignani et al., 2013; Dayan, Censor, Buch, Sandrini, & Cohen, 2013; Kuo & Nitsche, 2012; Miniussi, Ambrus, Walsh, & Antal, 2012; Stagg & Nitsche, 2011; Ziemann, 2004) depending on parameters which do not show any obvious link to intrinsic brain oscillations. These effects often manifest in cortical excitability changes. As posterior α-activity is taken to be an indicator of cortical excitability (Lange, Oostenveld, & Fries, 2013; Romei, Brodbeck, et al., 2008; Romei, Rihs, Brodbeck, & Thut, 2008), offline α-changes could reflect these forms of LTD and LTP (but see Veniero et al., 2011). In addition, overall metabolic or perfusion changes might be correlated with, and could possibly explain, excitability/α-changes (Antal, Polania, Schmidt-Samoa, Dechent, & Paulus, 2011; Laufs et al., 2003; Stagg et al., 2013). Such periodicity-independent LTD or LTP should occur to a similar extent for a broad range of stimulation protocols, such as reported for instance with repetitive TMS, where

LTD is associated with continuous low-frequency stimulation up to 1 Hz and LTP with interleaved or patterned high-frequency stimulation across many frequencies (5-20 Hz and iTBS) (Rossi, Hallett, Rossini, & Pascual-Leone, 2009).

Entrainment-dependent plasticity: The spike timing-dependent plasticity account

As introduced earlier, one mechanism that has been proposed to explain tACS-induced α-aftereffects is spike timing-dependent plasticity (STDP; Polanía et al., 2012; Zaehle et al., 2010). In STDP, the order and timing of pre-and postsynaptic potentials determine the magnitude, and direction, of changes in synaptic strength (Caporale & Dan, 2008; Dan & Poo, 2006; Feldman, 2012). Zaehle and colleagues (2010) used a neural network model incorporating STDP-rules to show that periodic 10 Hz-stimulation can strengthen or weaken the synaptic weights of neuronal circuits (recurrent loops) depending on their reverberation frequency. In this model, online entrainment is the window into longer lasting synaptic plasticity effects that translate into frequency-specific changes in oscillatory activity. The model predicts synaptic strengthening in dominant (α-)loops when the stimulation frequency falls into a narrow range of frequencies slightly lower than the spontaneous α-peak. It must be emphasised that the analogy between the modelled predictions and our empirical results is based on a number of assumptions, including that 10 Hz spiking activity (as modelled by Zaehle et al.) is driven by a 10 Hz alternating current, and that the synaptic strengthening of the responsive recurrent loops leads to an increase in natural α-activity. If these assumptions hold, this model matches our data, which show that slower stimulation (relative to IAF) enhances oscillations in the individual alpha (here: faster) frequency.

Importantly, this model not only predicts synaptic strengthening for stimulation with slightly slower frequencies, but also synaptic weakening in neural circuits when stimulation is applied at slightly faster frequencies relative to the intrinsic frequency. This parallels classical STDP models in which synapses are strengthened when the postsynaptic potential (here: spiking of the driving neuron at tACS frequency) follows the presynaptic potential (here: the feedback to the driving neuron via the recurrent loop), and weakened when the order is

reversed. This prediction can be verified experimentally, and was tested in the following experiment (Chapter 3).

Limitations of this study

First, the current design did not entail a condition with continuous stimulation, precluding a direct comparison between continuous and intermittent tACS aftereffects. It is therefore conceivable that continuous, but not intermittent, tACS leads to lasting entrainment given that in a typical tACS-protocol the brain oscillators are subjected to prolonged phase alignment over thousands of cycles. However, oscillatory phase in EEG recordings is generally instable over time, and as our data show, does not outlive tACS offset for more than 100 ms, thus supporting our conclusion that the aftereffect is predominantly a consequence of plastic changes.

Second, due to the lack of an effective tACS artefact removal method, we have no information about processes online to tACS. Nonetheless, there is growing evidence that entrainment during tACS is likely (Helfrich, Schneider, et al., 2014; Neuling, Rach, et al., 2012; Riecke, Formisano, et al., 2015; Riecke, Sack, et al., 2015; Ruhnau, Neuling, et al., 2016), and as discussed above,may even be a prerequisite for plasticity effects. In line with this view, Helfrich and co-workers (Helfrich, Schneider, et al., 2014) found that participants with greater α-power during tACS – interpreted as stronger entrainment - also tended to show greater aftereffects. Additional support comes from Neuling and co-workers, who followed up their finding of state-dependent alpha power aftereffects after tACS with eyes open that were not observed after stimulation with eyes closed (Neuling et al., 2013; Ruhnau, Neuling, et al., 2016). Using simultaneous tACS-MEG, they found that tACS at IAF elicited (online) signs of alpha entrainment in visual cortex only when participants had their eyes open but not closed. The relationship between online and offline effects is an important issue that requires the further development of artefact correction methods and may benefit from concurrent tACS-MEG measurements (Neuling et al., 2015; Ruhnau, Neuling, et al., 2016; Witkowski et al., 2016) that, at least at the source level, appear to be less sensitive to distortion than concurrent EEG (but see Noury et al., 2016).

Third, as in previous studies (Helfrich, Schneider, et al., 2014; Neuling et al., 2013; Neuling, Rach, et al., 2012; Zaehle et al., 2010) comparisons to control tACS frequencies are missing. Accordingly, it is unclear how frequency-specific the aftereffects are, although some insight on frequency-specificity can be derived from the observed variability in individual α-frequency with respect to a constant tACS frequency, with aftereffect magnitude being relatively unaffected by frequency mismatch. However, here the size of the mismatch was overall relatively small, and future studies need to clarify whether deviations (small or large) make a difference to outcomes. Moreover, we stimulated below, rather than above IAF. In the light of the STDP model, it will be interesting to determine if the direction of a (small) mismatch has a qualitative influence on the direction of the induced changes. This question is partly addressed in Experiment 2.

Lastly, there is no data available whether the observed quantitative change in α-power has any functional significance. This needs to be tested through additional behavioural manipulations pre versus post-tACS. For instance, positive aftereffects on cognitive (specifically: memory) performance have been reported after tSOS (Marshall et al., 2006; Veniero et al., 2015) and theta-tACS (Jaušovec & Jaušovec, 2014; Pahor & Jaušovec, 2014; Vosskuhl et al., 2015). It is unclear whether the behavioural changes found after tSOS sleep interventions have a direct oscillatory neural correlate as the behavioural post-tests in sleep interventions are typically taken long after stimulation and the acquisition of neural measures, and therefore probably reflect the product of some earlier interaction with brain activity (e.g., during memory encoding) rather than ongoing rhythmicity. Vosskuhl and colleagues (2015) did not acquire neural activity measures during working memory task performance, therefore not allowing assessment of brain-behaviour correlates of their tACS effect. While the group of Jaušovec observed changes in the theta and alpha bands, the precise nature of which depended on tACS montage, they did not specify whether these changes were in any systematic relationship to working memory performance (Jaušovec & Jaušovec, 2014; Pahor & Jaušovec, 2014). The existing evidence for lasting behavioural changes is scarce; therefore, an important step on the way towards a viable therapeutic intervention will be to show that tACS can affect both brain activity and associated functions.

Conclusion

Offline α-enhancement after α-tACS reflects short-term neural plasticity rather than entrained activity, although it is likely that mechanisms set in motion by online entrainment are prerequisite to such effects. While the present data cannot disambiguate between the entrainment-dependent and independent plasticity accounts, they partially match the predictions of an STDP model. This model makes additional predictions that can be tested.

The presence of aftereffects beyond stimulation underlines the potential of tACS as a therapeutic tool. In addition, our findings may be informative for study-designs. Given that α-aftereffects were negligible with short trains (3 s) and participants overall tolerated the discontinuous stimulation well, intermittent event-related tACS paradigms with short trains could be viable tools in cognitive research on online tACS effects when potential confounds from aftereffects must be minimised.

Chapter 3. Aftereffects are not replicated testing the spike timing-dependent plasticity hypothesis of α-tACS-induced alpha power enhancement (Experiment 2)

To quickly recap, the previous experiment established that intermittent tACS at alpha (α-)frequency can induce lasting changes in α-activity (aftereffects) as demonstrated by an increase in α-power. These changes did not show the characteristics of entrained oscillations. This suggests some form of tACS-induced plastic mechanism at the neural network level. A computational model for a mechanism based on spike-timing dependent plasticity (STDP) has been developed by Zaehle and co-workers (Zaehle et al., 2010), which will be reviewed in more detail in the following paragraphs (see also Figure 3.1). The model invites certain predictions with regard to the relationship between frequency of stimulation and the expected changes of network activity. Specifically, it can be hypothesised that spontaneous α-activity will be enhanced by α-tACS at or just below the endogenous individual alpha frequency (IAF). In contrast, α-tACS above the IAF should have no effect or even suppress spontaneous alpha. The current study tested these predictions.

In the STDP framework, the strengthening (long-term potentiation, LTP) or weakening (long-term depression, LTD) of a given synapse depends on the relative timing between presynaptic and postsynaptic activity (Figure 3.1A, based on Bi & Poo, 1998; Bi & Wang, 2002; for a review of the different types of STDP models see Feldman, 2012). The classic STDP model in its simplest form (consisting of one synapse between two excitatory neurons connected in sequence) predicts synaptic LTP when the pre-synaptic potential precedes the post-synaptic potential within a brief time window, implying a causal chain between a stimulus and its associated response. LTD occurs when the pre-synaptic potential follows the post-synaptic potential, conceptually describing a response that is independent of the stimulus.

In order to explain their α-aftereffects following α-tACS, Zaehle and colleagues (2010) implemented a simplified two-layer model with a single excitatory input neuron driving a hidden layer of excitatory spiking neurons at a

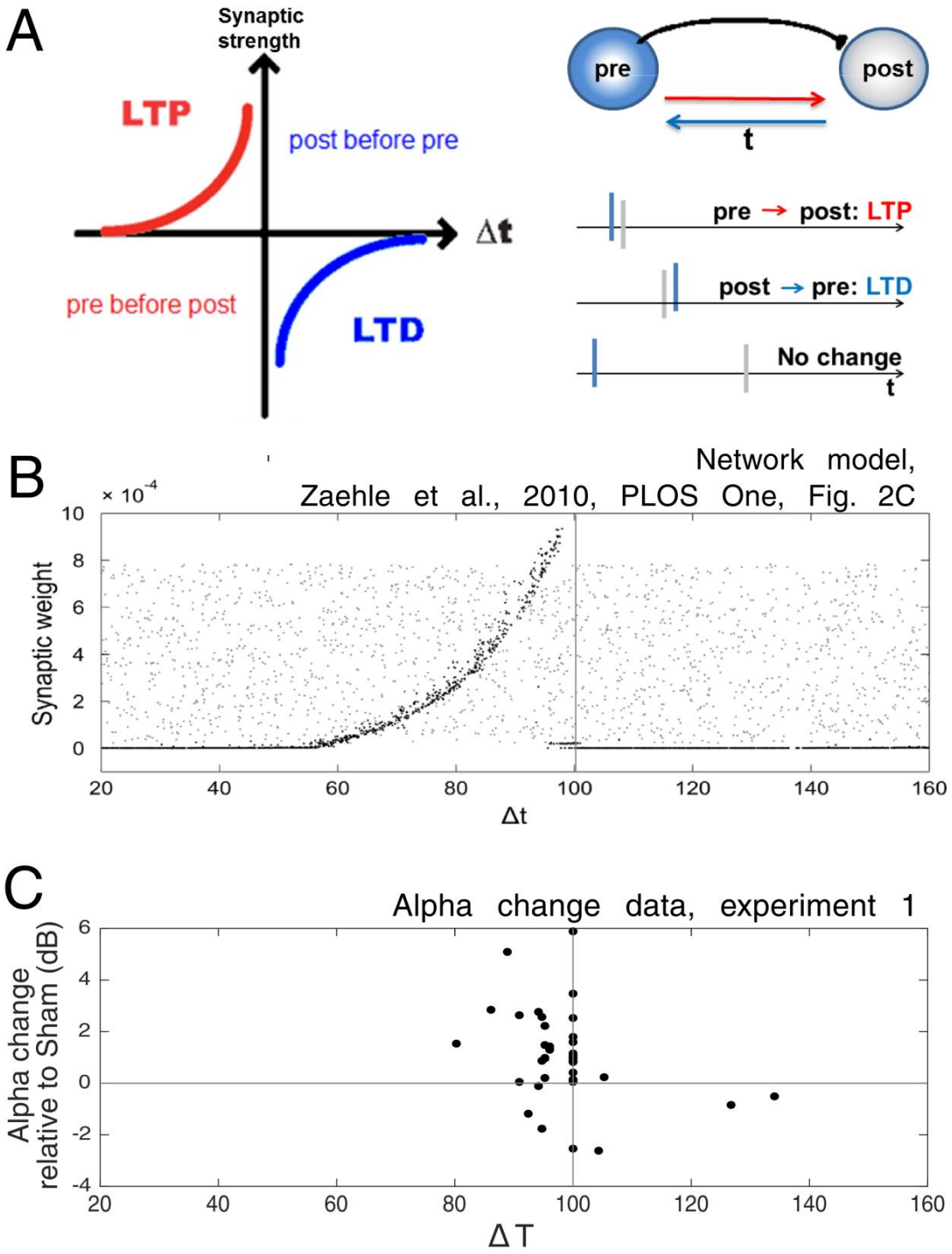

Figure 3.1: Spike timing-dependent plasticity (STDP) model of tACS.

A) Classic model of STDP. When the postsynaptic potential follows the presynaptic potential within a brief time window, this synapse will be strengthened. When the postsynaptic potential precedes the presynaptic potential within a brief time window, this synapse will be weakened. Without temporal proximity, no synaptic changes occur. *B)* Network simulation results from Zaehle et al.'s STDP model of tACS aftereffects (see also Zaehle et al., 2010, their Figure 2C; graph reproduced with the authors' permission). *C)* Alpha power enhancement as observed in Experiment 1 (see Figure 2.6C), re-expressed as a function of the period of individual alpha frequency relative to that of the stimulation frequency (as T = 1/f, here for ease of comparison aligned to 100 ms).

rate of ten spikes per second (10 Hz), i.e., with a period of 100 ms between successive spikes. The model was set up as follows. Each neuron in the in the hidden layer was connected to the driving neuron in a feedback loop. Considerations of synaptic plasticity refer to the strength of the back-projections onto the input neuron. The delay of recurrent feedback within each loop was randomly drawn from a uniform distribution of delays between 20 and 160 ms. In terms of oscillatory period, this corresponds to frequencies between 6.25 and 50 Hz. Conceptually, the feedforward spiking neuron entrained a given downstream neuron, whose activity was 100% phase-locked to the 10 Hz rhythm, albeit with a constant phase offset depending on the specific delay in that loop. Synaptic weights for feedforward and feedback connections were drawn from (different) uniform distributions, with higher weights for feedforward connections. No connections were modelled between neurons in the hidden layer.

This simulation resulted in enhanced synaptic weights for feedback loops with a total period (or delay) slightly shorter than one inter-spike period of the input neuron, while for much shorter as well as longer periods those weights were reduced (Figure 3.1B). In other words, in feedback loops resonating at a frequency slightly higher than the feedforward input frequency of 10 Hz LTP occurred if the feedback potential (here conceptually equivalent to a presynaptic potential, or S1[1]) preceded the feedforward spike (by extension the postsynaptic potential, or S2) roughly between 0 and 40 ms. In contrast, for loops in which feedback arrived outside this range LTD was observed. (Note that due to the rhythmicity of the simulated input signal, for a loop with a cycle length greater than the inter-spike period the feedback potential S1 would arrive only in the next inter-spike period, i.e., follow the subsequent spike S2.)

The authors suggested that their results can be translated to the observed tACS-induced α-power enhancement in their EEG study with human observers, through the stabilisation of feedback loops supporting α-activity by α-tACS. In

[1] I realise that it is not intuitive to think of a feedforward signal as post-synaptic and the associated feedback signal as pre-synaptic, despite the circular nature of the feedback loop and the specific synapse that is considered here (between the back-projection of the driven feedback neuron and the driving feedforward neuron). As a mental crutch, one can ignore the feedforward connection for the moment and imagine that the feedback neuron is driven by a different external force to better visualise how STDP might act.

this conceptual model, online entrainment is therefore posited as a window into longer-lasting synaptic plasticity that translates into frequency-specific changes in oscillatory activity. Its predictions were partly met by the results in experiment 1 (see, Figure 3.1C, also Figure 2.6C) where, following stimulation with an intermittent eight second on/off pattern at a frequency slightly lower than the participant's IAF, α-power was, on average, enhanced relative to sham, consistent with the idea of LTP of α-circuits. However, Zaehle and co-workers' results also imply a potential directionality of the plastic change (LTP versus LTD) which depends on the sign of the relative mismatch between the period of the driving rhythm and the latency of the feedback response. An interesting question is whether this hypothetical directionality also translates to mismatch-dependent power increases or decreases in the α-band and other physiologically relevant frequencies. The differential up- or downregulation of synchronous brain activity could prove an exciting, non-invasive tool in interventions for disorders associated with abnormal neural synchrony, such as Alzheimer's disease, Parkinson's disease, and schizophrenia (Schnitzler & Gross, 2005; Uhlhaas & Singer, 2006).

In their work, Zaehle et al (2010) modelled the initial strength of the back-projections from a randomly drawn uniform distribution of synaptic weights. This implies that the (initial) feedback connectivity is unbiased towards specific resonance frequencies. However, it seems physiologically plausible that in the presence of a dominant frequency (specifically, a person's dominant posterior α-frequency), selective feedback loops with certain time constants should possess higher starting weights than "weak" frequencies, and should be more amenable to plastic changes both in terms of LTD (because less prone to floor effects) and LTP (because of overall higher activity in this loop). In other words, we would expect greater effects at the resonance frequency of a person's dominant circuit when stimulated at nearby frequencies, but lesser or no effects at non-dominant frequencies. For instance, in a participant with a 10 Hz α-peak, aftereffects would predominantly be observed at this intrinsic 10 Hz frequency after stimulation at a nearby frequency, but no aftereffects should be observed at non-dominant frequencies (e.g., 7 Hz) with stimulation near these frequencies (nor should there be 10 Hz aftereffects after 7 Hz stimulation, which would be too great a mismatch to yield effective pre-post

synaptic pairing.). Hence, although Zaehle et al.'s model is motivated by the existence of intrinsic resonance frequencies, it does not explicitly take them into account. With the new assumption of biased starting weights, the model predicts synaptic strengthening in dominant (α-)loops only (or at least predominantly) when the stimulation frequency falls into a narrow range of frequencies slightly lower than the spontaneous α-peak.

This idea is visualised in Figure 3.2. Consider two neurons arranged in a feedback loop (Figure 3.2A). One is driven by tACS, i.e., this neuron is sufficiently stimulated that its activity is entrained by the electric current. This neuron can be considered the equivalent of the feedforward neuron in Zaehle et al. As it is postsynaptic to the back-propagating neuron we will call events at its synapse S_{post}. The other neuron may be partially driven by tACS but is not stimulated sufficiently to fully phase align its activity with the other neuron. Hence, events at the back-propagating synapse (which we will call S_{pre}, as they occur presynaptic to the feedforward neuron) do not occur simultaneously (that is, entirely driven by tACS and independent of the feedforward signal) but arrive at or near its natural delay.

If the dominant (α-)frequency in this feedback loop is slightly higher than the stimulation frequency (and the cycle duration is shorter), postsynaptic events (S_{post}) driven by tACS are generated at a slightly slower pace than the time required for the feedback (S_{pre}) through the dominant recurrent loops (resonating at IAF). As a consequence, presynaptic (feedback) events have a higher likelihood to slightly precede the post-synaptic events in these loops (see Figure 3.2B, bottom), leading to strengthening of their associated synapses. Conversely, synaptic weakening in dominant α-loops is predicted when stimulation is applied at slightly faster frequencies relative to the spontaneous α-peak frequency (Figure 3.2C). This parallels classical STDP models in which synapses are strengthened when the post-synaptic potential (here: spiking of the driving neuron at tACS-frequency) follows the pre-synaptic potential (here: the feedback to the driving neuron via the recurrent loop), and weakened when the order is reversed.

It must be emphasised that this model is based on a number of assumptions (see also Zaehle et al., 2010). The first assumption is that 10 Hz

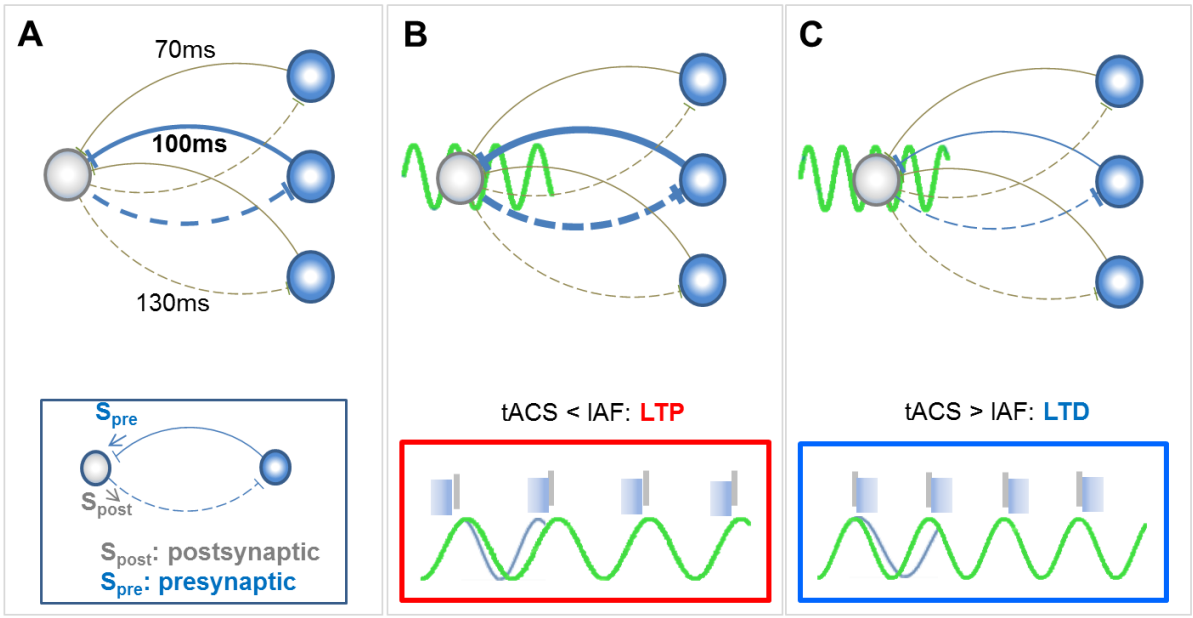

Figure 3.2: Extended conceptual STDP model of tACS aftereffects

A) Recurrent loops in a population of neurons oscillating at alpha frequencies reverberate at different delays, leading to a net oscillatory frequency depending on which connection dominates. Dominant frequency can slowly fluctuate over time/days. In this example, delays of 100 ms dominate, leading to a dominant 10 Hz oscillation. *B) and C).* Stimulation by tACS. Some neurons are strongly modulated by tACS (grey circles) while others are not (blue circles) (the influence of tACS is unlikely homogeneous across neuronal tissues and locations). Consider the synapse on the grey neurons. Events are triggered rhythmically by tACS (S_{post}, assuming action potential generation shaped by stochastic resonance (McDonnell & Abbott, 2009). These events occur in close temporal proximity with feedback events, S_{pre}, resonating through recurrent loops at the delay of the dominant cycle (here 100 ms). *B)* When neurons are stimulated at a frequency slightly slower than the dominant frequency of the loop (i.e., IAF), presynaptic events tend to slightly precede postsynaptic events of the next cycle, leading to strengthening of the synapse (LTP). *C)* Conversely, when neurons are stimulated at a frequency slightly faster than the dominant frequency, presynaptic events tend to slightly follow postsynaptic events of the next cycle, leading to weakening of the synapse (LTD).

spike bursts result from a 10 Hz alternating current. In contrast to TMS, which can elicit action potentials directly, electrical stimulation methods such as tACS and tDCS are known to act on a subthreshold level by modulating the resting membrane potential (Fertonani & Miniussi, 2016). However, a higher rate of neural spiking activity is likely due to the process of stochastic resonance, where the addition of electrical noise can increase the probability that a given neuron will become sufficiently depolarised to discharge (de Berker, Bikson, & Bestmann, 2013; McDonnell & Abbott, 2009; Miniussi, Harris, & Ruzzoli, 2013). Second, it assumes that the synaptic strengthening (or weakening) of recurrent loops with the cycle length of an α-period leads to an increase (or decrease) in natural α-activity. To my knowledge, this has not yet been established empirically. Third, it assumes that entrainment through α-tACS is only partial. During complete entrainment, the neural population activity would phase-align

completely to the stimulation frequency; that is, any activity would be confined to a small simultaneous window. For STDP based on small but systematic differences in the timing of activity, the population of downstream neurons should be at least partly independent and capable of escaping the overall drive by tACS. If a fraction of the targeted neurons can escape entrainment at any given instance, an overall bias towards the dominant frequency should develop which is – depending on the dominant frequency - either faster or slower than the tACS frequency. This bias is what can give rise to the temporal offset required for plasticity. Under synchronisation-theoretical considerations, partial entrainment is expected around the edges of an Arnold tongue (Fröhlich, 2015). Given that cortical neurons are massively interconnected, it is improbable that network activity will be completely dominated by weak external stimulation, and such partial entrainment is plausible.

If these assumptions hold, this model matches the data of Experiment 1, which show that slower stimulation (relative to IAF) enhances oscillations in the individual (here: faster) α-frequency. That said, the frequency offset to IAF was essentially accidental. Therefore, the data set provided no information about the potential effects of tACS applied at frequencies slightly above the dominant frequency, specifically, whether this would lead to LTD in the form of decreased α-activity relative to sham. The objective of this experiment was to find evidence for or against the STDP account by studying the relative changes in α-power as a function of the sign of the frequency mismatch. Specifically, our aims were, firstly, to replicate the finding of α-enhancement in Experiment 1 by stimulating with intermittent tACS at a frequency just below IAF; and secondly, to test the STDP model predictions by also stimulating intermittently slightly above IAF. To improve on the limitations of the previous experiment, a continuous control condition was included to assess the efficacy of intermittent tACS compared to continuous stimulation (as in Helfrich, Schneider, et al., 2014; Neuling et al., 2013; Zaehle et al., 2010). It was hypothesised that *i)* lower-frequency intermittent and continuous tACS would be followed by α-enhancement (relative to sham), thus replicating previous observations and *ii)* in line with the opposing predictions of our STDP model, higher-frequency tACS would either be followed by a relative α-weakening, or show no difference from sham.

Table 3.1: Participant demographics and stimulation parameters

IAF = Individual alpha frequency (as determined from pre-test EEG on the first day of testing); mA/pp = milliampere peak to peak.

ID	*Sex*	*Age*	*IAF*	*Intensity (mA/pp)*
02	f	24	10.50	2.00
06	m	22	10.75	2.00
08	m	23	10.00	2.00
09	f	19	10.25	2.00
12	f	19	11.75	1.70
13	m	28	9.75	1.70
14	f	25	10.75	1.65
16	m	20	10.00	2.00
17	m	25	9.50	2.00
18	m	24	9.50	1.75
19	m	24	11.25	1.80
20	f	22	9.50	1.75
21	f	18	10.50	1.55
22	f	20	10.25	1.80

Methods

Unless stated otherwise, the apparatus and methods were the same as in Experiment 1. Please refer to the previous chapter.

Participants

Twenty-three healthy participants were recruited from colleagues and the department's subject pool. Acceptable data for all four conditions were recorded for fourteen volunteers (seven male, age range 18 - 28 years, *M* = 22.9, *SD* = 2.7). Of the remaining nine participants, two did not return to their last session; one had excessive noise in one condition and was unavailable for an additional session; four did not show enough discernible α-activity in their first session to determine the stimulation frequency; one could not tolerate the discomfort induced by tACS; and one was invited but excluded after reporting excessive recent recreational drug use during screening in the first session. Incomplete data sets were not included in this analysis. The demographics and stimulation parameters are shown in Table 3.1.

tACS

The montage was identical to Experiment 1 with tACS electrodes over PO7/9 and PO8/10, respectively. There were three active conditions and a sham condition in counterbalanced order (Figure 3.3). Since in the previous study the long protocols were the most effective in inducing an aftereffect, a similar protocol was adopted for both intermittent tACS conditions. Unlike in the former experiment, however, tACS-frequency was not constant across sessions. To keep overall duration per condition identical, we therefore chose a constant eight second on/off protocol for all participants and frequencies (as compared to an eighty cycle on-off protocol, cf. Experiment 1).

To probe for directional STDP effects tACS was applied either at IAF - 0.75 Hz (*low intermittent condition*/*LowInt*, with lower stimulation frequency hypothetically strengthening dominant α-circuits) or IAF + 0.75 Hz (*high intermittent condition*/*HighInt*, with higher stimulation frequency hypothetically weakening dominant α-circuits). In order to assess the efficacy to produce aftereffects of intermittent versus continuous stimulation, a *low continuous condition*/*LowCont* was added in which continuous tACS was administered without stimulation-free intervals only during the second half of the experimental session. We decided against counterbalancing (i.e., continuous stimulation in the first versus second half) because of the likely decay of the aftereffect before the post-test recording. The "low intermittent" frequency was used also for the continuous condition as it allows direct comparison with the low intermittent protocol, and because lower-than-IAF stimulation proved to be successful in Experiment 1. The *sham condition*, during which only a brief (20 s) stimulus was applied at the beginning, controlled for stimulation-unspecific effects such as changes in arousal.

tACS intensity was adjusted individually below phosphene- and discomfort threshold but constant across conditions for each participant, ranging between 1.55 and 2.00 mA (peak-to-peak amplitude; M = 1.84, SD = .16).

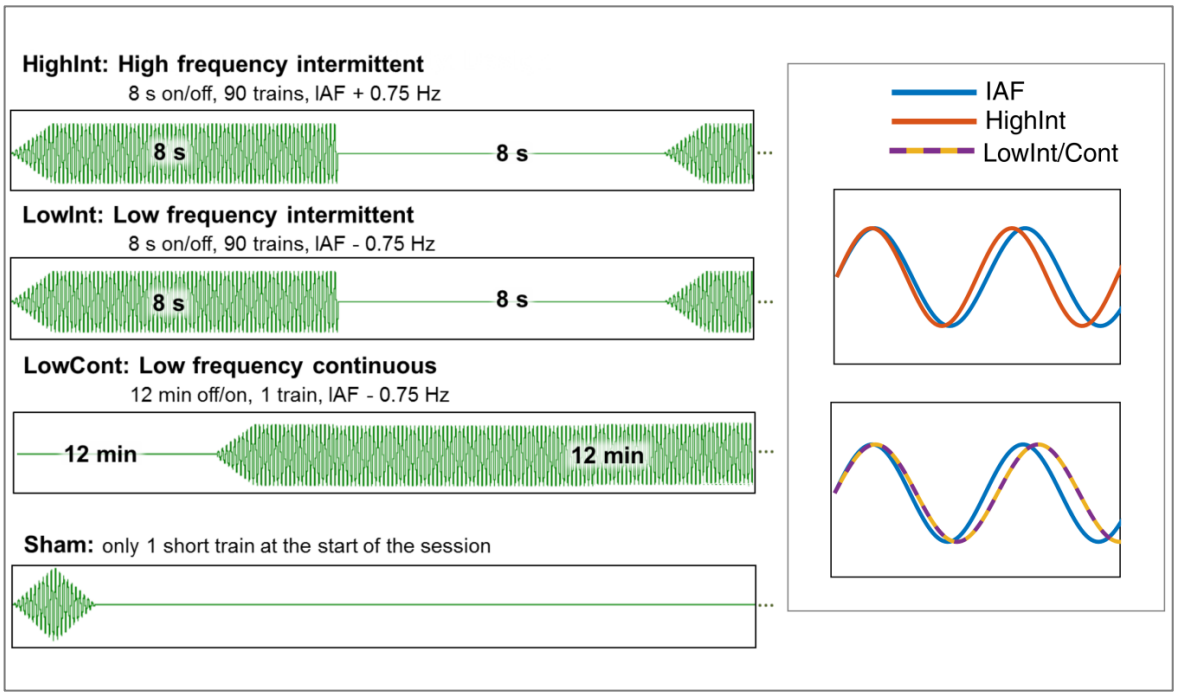

Figure 3.3: tACS protocols

Left: Across conditions, we manipulated the tACS frequency (individual α-frequency/IAF – 0.75 Hz or IAF + 0.75 Hz) and temporal pattern (intermittent or continuous. *Right:* Because of the expected offset between IAF and tACS frequency, endogenous oscillatory activity (blue line) is expected to "run away" or escape from the external drive by tACS.

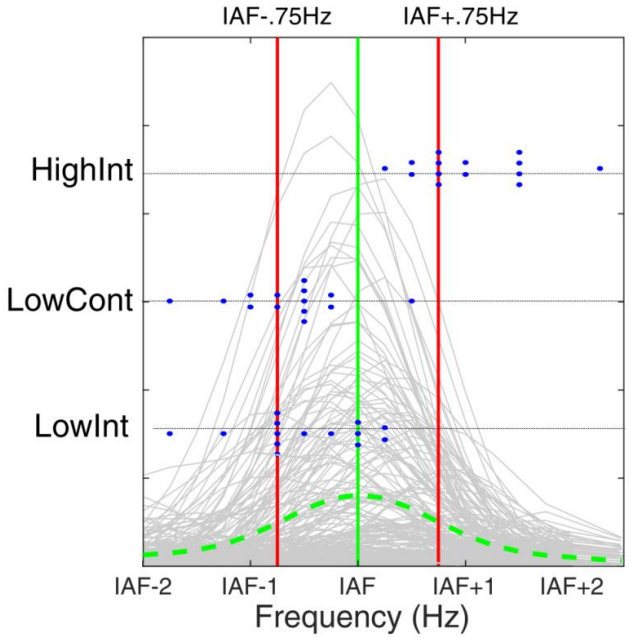

Figure 3.4: Idea of experimental design and evaluation of IAF estimation

Shown in light grey are single trial EEG spectra in the α-range for an individual with a discernible α-peak. Green dotted line represents the mean power spectrum across trials. The estimated individual α-frequency (= IAF in the pre-test of the first session) is marked by the green vertical line. Note also the inter-trial variability around this peak. Red vertical lines mark the targeted frequencies slightly below (IAF – 0.75 Hz) and above (IAF + 0.75 Hz) the peak frequency. Blue dots represent individual participants in each of the three active protocols (grouped along the grey horizontal lines) and the actual difference between IAF (estimated per session) and the tACS frequency of that session. Blue dots on the red vertical line of the corresponding condition therefore have been estimated most accurately. The difference between low and high frequencies is by definition 1.5 Hz.

To assess whether the stimulation frequencies actually targeted the lower/higher tails of the individual α-power spectrum, we also determined the dominant α-frequency for each session individually, using a bootstrap algorithm (see section *Individual alpha frequency* below) on artefact-free data recorded from electrode POz and frequencies between 8 - 12 Hz. Day-to-day IAF was defined as the mode of the peak frequencies of each bootstrap sample. Figure 3.4 shows the estimated differences between the IAF on the day of a given session and the respective stimulation frequency.

Procedure

Each participant underwent four sessions of maximally two hours each. Sessions were at least three days apart. Preparation of tACS and EEG electrodes took approximately 45 min and the recording around one hour. Data acquisition started with resting EEG with eyes closed (3 min) and eyes open (5 min) (pre-test). Note that longer resting EEGs were taken to ensure a minimum of 120 s, as previous work indicates as the required minimum to obtain a stable spectral estimate (Brismar, 2007), and to be able to assess the duration of a possible aftereffect. Participants then underwent one of the stimulation protocols in counterbalanced order. For the duration of each protocol, participants performed a visual vigilance task to maintain alertness and as control for cognitive state (see Experiment 1). Finally, additional resting EEGs with eyes open (5 min) and eyes closed (3 min) were recorded (post-test). After each session, participants filled in a questionnaire with visual analogue scales (VAS) to assess how intensely they perceived itch ("Itch"), discomfort/pain ("Discomfort"), or visual sensations ("Visual") due to tACS. Items were rated on a scale on a scale from 0 to 10 where 0 is "no sensation" and 10 is "very strong sensation" (see Appendix B for post-test questionnaire).

EEG recording

Recordings were obtained from eight posterior-parietal scalp locations (C3/4, Cz, P3/4, Pz, POz, Oz, ground AFz, referenced to the left mastoid, except one recording with nasion reference) according to the 10/10 system using a BrainAmp MR Plus amplifier (BrainProducts, Munich, Germany) and sintered Ag/AgCl electrodes. (Initially, we recorded from electrodes across the whole

scalp mounted in an electrode cap. However, the tACS electrode sponges tended to soak the fabric over time. This created bridges to adjacent EEG electrodes which then in turn were contaminated by high amplitude electrical noise, necessitating the re-recording of one data set and resulting in incompleteness of another. Therefore, EEG electrodes were attached as previously using EC2 electrode paste. However, to keep the preparation time constant, coverage was restricted to the above-named positions.) Vertical eye movements were recorded from an additional electrode below the right eye. The signal was amplified to a range of ±3.2768 mV at a resolution of 0.1 μV, bandpass-filtered online between 0.1 - 1000 Hz and digitised at a sampling rate of 5 kHz.

Individual alpha frequency

Individual alpha frequency (IAF) and stimulation intensity were determined once, in the first session, for all four sessions. Because of the variability in α-peaks in Experiment 1, it was attempted to make the estimate more reliable by 1) removing epochs with eye blinks, 2) removing epochs with unusual broadband spectral activity, 3) using algorithm-guided visual inspection (see below).

The continuous EEG acquired at rest with eyes open was segregated into 1 s epochs. Epochs containing eye blink artefacts were identified in the EOG channel using the automatic artefact rejection algorithm as implemented in FieldTrip (Oostenveld, Fries, Maris, & Schoffelen, 2011). The signal was first bandpass filtered between 1 and 15 Hz to optimise blink detection and z-transformed, and then epochs containing z-values of greater than |3| were discarded from the unfiltered data set. The blink-free epochs were Fourier-transformed (2 - 20 Hz, Hanning window, 4 s zero padding, 0.25 Hz resolution). Spectra with unusually high mean power (z-transformed power > 2) were removed. Note that the mean power calculation in this step explicitly excluded the α-band between 6 and 14 Hz, as trials with very high α-power easily yield a high mean power but being the trials of interest should not be discarded.

Then the posterior channel (i.e., of Pz, POz, and Oz) with the highest mean power was selected for a bootstrap procedure (2, 12, and 1 participant, respectively). Fourier spectra were randomly selected with replacement from

the number of available spectra (N_s) to form bootstrap samples of the same size N_s. For each bootstrap sample, the peak frequency of the median power spectrum in the frequency range between 7 and 13 Hz was calculated (median rather than mean to relieve the influence of few high power trials) and stored. This was repeated 1000 times. The mean, mode, and median of the bootstrapped frequency maxima were calculated and compared. If the peak frequency estimates agreed, this frequency was chosen as IAF. If they disagreed (e.g., because of a bimodal bootstrap distribution or high peak variability) the IAF was estimated based on visual inspection of the single trial spectra. IAF ranged from 9.50 - 11.75 Hz (M = 10.30, SD = .67) across participants (see Table 3.1).

EEG analysis of offline effects

The analysis was similar to the one described in Experiment 1. Pre- and post-test EEGs were re-referenced offline to electrode Cz. After epoching into 1 s epochs and artefact removal, the first ten of the remaining epochs were discarded to allow for the signal to settle. A Fast Fourier Transform (4 - 30 Hz, Hanning window, 4 s zero padding, 0.25 Hz resolution) was calculated on the following 120 epochs (2 min) of each set. Alpha power was calculated from the resulting spectra as the mean power across trials and across frequencies in the individual α-band (IAF - 2 Hz to IAF + 2 Hz) for all pre- and post-tests in each condition. Alpha power change from pre-test to post-test was defined as

$$Change = 10*log(post\text{-}test/pre\text{-}test)$$

(in dB) within each condition. A nonparametric Friedman test for the main effect of tACS condition was performed on the relative power change at electrode POz.

Robust regression

For exploratory analysis of the association between the effect of tACS and different variables, the strength of the correlation was tested statistically using Spearman's rank correlation. If the data and test suggested a trend towards a monotonous relationship, we additionally calculated Shepherd's *pi* (Schwarzkopf, De Haas, & Rees, 2012). The Shepherd's *pi* correlation involves the identification and exclusion of influential points before the calculation of Spearman's *rho*. In other words, *pi* corresponds to Spearman's *rho* with outliers removed and is

identical when no influential points are present. These two statistics in combination should give a reasonable idea of the strength of the association between two variables, and its dependence on subsets of participants.

Results

Peripheral sensations

The distribution of participants' ratings of peripheral sensations per condition and type is shown in Figure 3.5. Notably, this group of participants generally reported higher sensations compared to Experiment 1 (compare Figure 2.10A in the previous chapter). This is true for both the active and sham conditions.

To assess whether stronger sensations were perceived during active tACS compared to sham, the VAS scores for Itch, Discomfort, and Visual sensations were submitted to separate Friedman tests. The tests for Discomfort ($X^2(3) = 2.71$, $p = .44$) and Visual ($X^2(3) = 1.17$, $p = .76$) were not significant, indicating that active tACS did not systematically induce painful or visual sensations across participants relative to sham. The test for Itch was marginally significant ($X^2(3) = 7.98$, $p = .047$) but follow-up Wilcoxon Signed rank tests indicated that this effect was driven by the difference between the continuous and intermittent conditions (LowCont versus LowInt: $Z = -2.48$, $p = .013$; LowCont versus HighInt: $Z = -1.88$, $p = .061$, all other comparisons $p > .29$). This makes sense intuitively as there are far more stimulation on- and offsets that can induce itchy sensations but as none of these conditions appears to induce greater sensation than sham overall, it is inconclusive whether the itchy sensation is due to electrical stimulation or simple due to the contact between the skin and the moist sponges.

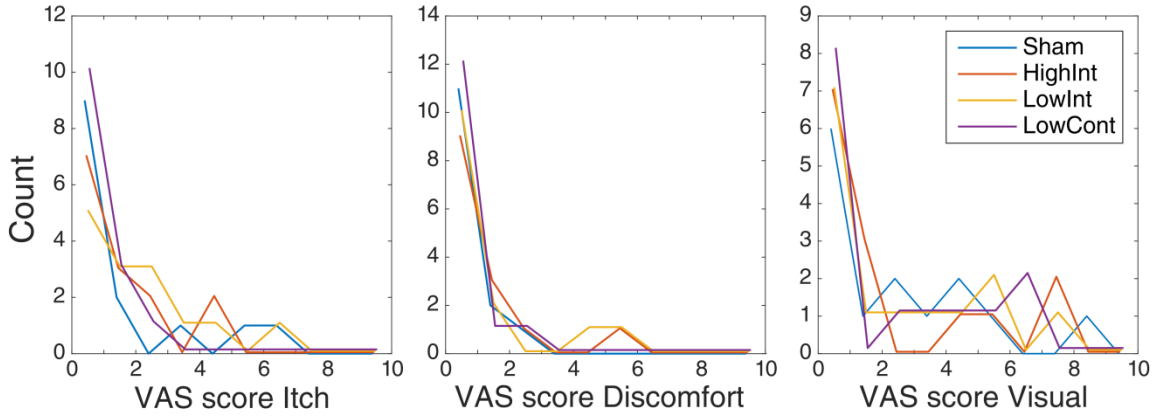

Figure 3.5: Histograms of Visual Analogue Scale scores

The histograms represent the distribution of VAS ratings of the presence and intensity of peripheral sensations in the different conditions. Zero represents no sensation, ten represents strong sensation.

Alpha power: No systematic absolute or relative changes

Inspection of the individual responses shows that participants did not respond uniformly to any of the active protocols relative to sham (Figure 3.6, top right), or show systematic differences between the active tACS protocols (Figure 3.6, bottom right). Accordingly, α-power changes were not statistically discernible at the group level between tACS conditions ($X^2(3) = 3.34$, $p = .34$; Figure 3.6, top and bottom left). The strongest increase was expected for intermittent stimulation at IAF - 0.75Hz (LowInt tACS). However, numerically (although not statistically), α-increase was on average stronger after intermittent tACS at IAF + 0.75Hz (HighInt) compared to all other conditions, opposite to the hypothesised direction (exploratory pairwise comparisons all $p > .05$; see also Figure 3.6 top and bottom left). Moreover, contrary to our expectation of a robust increase after continuous stimulation at IAF - 0.75Hz (LowCont), this protocol turned out to be the least effective in producing α-increase from pre- to post-test (HighInt: 13 out of 14 participants, LowInt: 12/14, LowCont: 9/14; sham: 10/14; see Figure 3.6 bottom left).

In sum, the data do not support the suggested STDP model of α-aftereffects. Moreover, the experiment also failed to replicate the differential α-power enhancement after intermittent tACS (as opposed to sham) under very similar conditions, with equivalent power (12 versus 14 participants) and using the same analysis as in Experiment 1. Finally, continuous tACS, which was

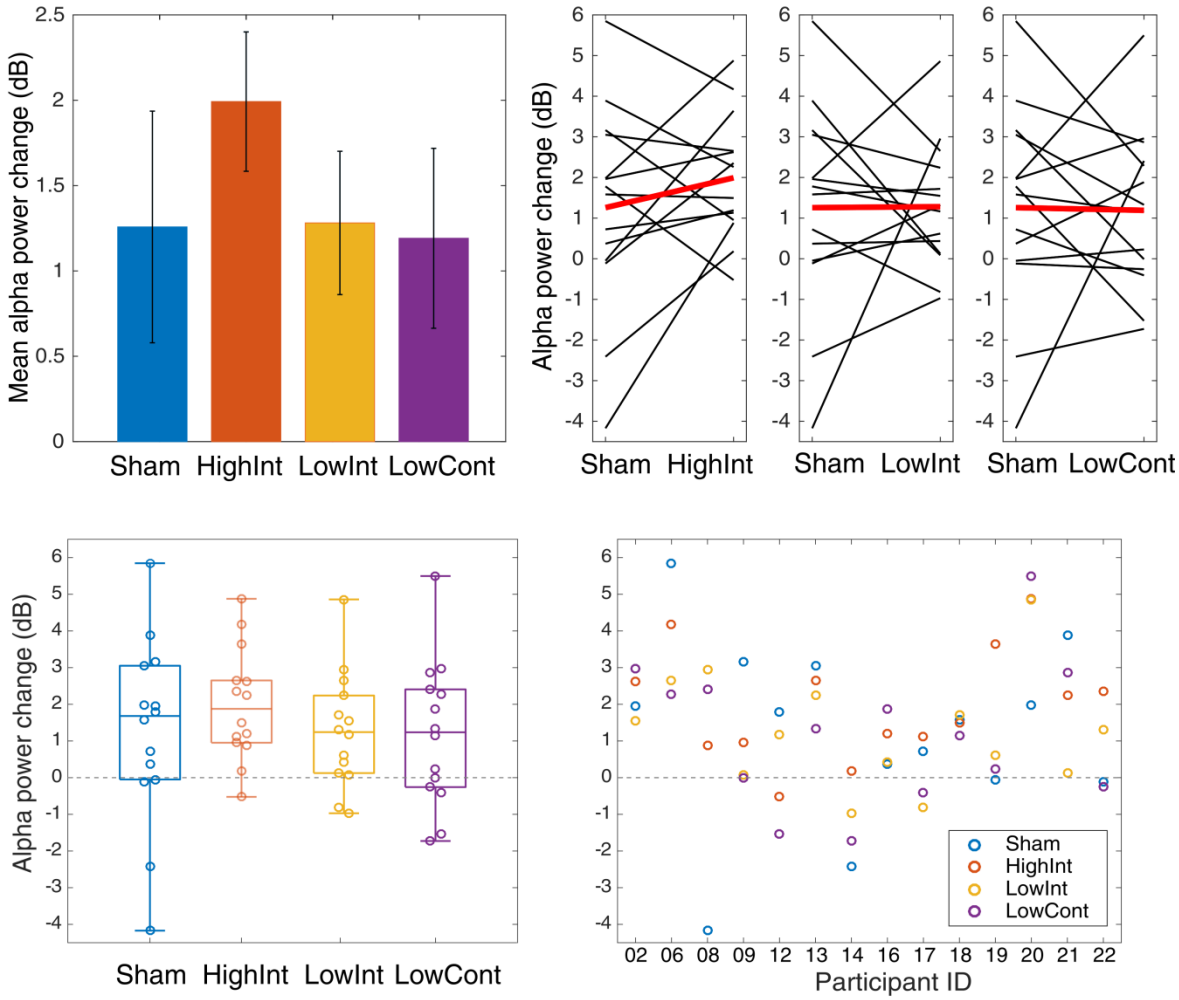

Figure 3.6: Change in alpha band power

Top left: Grand mean change in resting EEG power (in dB) within the individual alpha band (individual α-frequency determined on day 1 ± 2 Hz) from pre-test to post-test with eyes open. Error bars represent standard error of the mean (*N* = 14). *Top right:* Individual α-change contrasting sham versus active tACS conditions. Red lines represent group mean changes. *Bottom left:* Circles represent individual α-change scores grouped per protocol. Boxes show 25/50/75th percentile, whiskers enclose 1.5 * interquartile range. *Bottom right:* Same data but now rotated and grouped per subject. Grey dotted lines in both plots represent no change.

successfully used to enhance α-power in a number of previous studies (Helfrich, Schneider, et al., 2014; Neuling et al., 2013; Zaehle et al., 2010), did not produce a systematic increase in α-activity that was distinguishable from sham.

Control analyses: Influence of reference electrode

In this study, the EEG was referenced to Cz (as opposed to AFz in the previous experiment). As the reference is closer to the (posterior) site where we expect the tACS-induced changes, it is possible that these activities contaminated the reference and were consequently subtracted from the target electrode signal. To assess this possibility, the data from Experiment 1 were re-

referenced to Cz and re-analysed. The results for the main effect of tACS protocol were statistically equivalent to the EEG data referenced to AFz (please refer back to the results section in Chapter 2): The Friedman test with factor tACS protocol was significant ($X^2(3) = 8.1$, $p = .044$), and the follow up tests showed significant differences only between either of the two long conditions and sham (LongCo versus sham: $Z = 2.67$, $p = .008$; LongDis versus sham: $Z = 2.20$, $p = .028$; all other comparisons $p > .18$).

The data from Experiment 2 were also analysed at Oz, which has a greater distance from the reference as POz but a comparable distance from the tACS electrodes. Still, there was no main effect of tACS protocol ($X^2 = 4.71$, $.p = .19$). Taken together, this makes it unlikely that the proximity to the reference electrode overshadowed the effect.

Exploratory analyses: Idiosyncratic response independent of protocol?

Inter-individual differences in the response to stimulation protocols are a known problem in brain stimulation studies (Benwell, Learmonth, Miniussi, Harvey, & Thut, 2015; Fertonani & Miniussi, 2016; Wiethoff, Hamada, & Rothwell, 2014; Ziemann & Siebner, 2015). Despite the lack of a consistent group effect, it is possible that tACS induces idiosyncratic but potentially opposite responses in individuals, and that only per chance the majority of responders in our previous (small) sample responded in a similar fashion. Therefore, we also looked at intra-individual variability of the response to α-tACS (independent of the specific type of protocol). An interesting observation suggested a generic, protocol-unspecific but reproducible effect of tACS within individuals: When comparing the relative changes in the active conditions and sham -

$$tACS\ effect = change_{activetACS} - change_{Sham}$$

- the majority of participants seemed to respond consistently either with relative α-power enhancement or suppression (Figure 3.7A). Specifically, five participants showed a consistent decrease in α-power change relative to sham across all active conditions, whereas another five participants showed a

consistent increase; those who were more variable in their response also tended to have the smallest differences.

In order to quantify the likelihood of each participant responding uniformly to tACS with either increase or decrease if tACS has no effect at all (that is, if we observe truly random fluctuations), we conducted a permutation analysis based on a test statistic derived from the whole data set, calculated as the sum of the absolute values of the mean change difference between each active protocol and sham. In more detail: first, the change in the sham condition was subtracted from the change in each active protocol to obtain three difference scores for each participant, one for each active condition; second, these difference scores were averaged to obtain one mean difference score per participant; third, the absolute values of these mean difference scores were summed across participants to obtain the test statistic. This summary statistic disregards the direction of change but is large when all values within an individual data set are either consistently positive or consistently negative, and smaller otherwise (i.e., for changes of 2, 3, and 4 dB, the absolute value of the mean is 3 dB; for -2, -3, - 4 dB the absolute value of the mean is also 3 dB; for 2, -3, 4 dB the absolute value of the mean is only 1 dB). In addition, it is larger when as a group more individuals show a consistent direction.

To test the likelihood of this statistic under the null hypothesis that sham is interchangeable for any other condition, we randomly re-shuffled the condition labels within participants. Then the newly assigned "sham" condition was subtracted from all the other conditions. Finally, the summary statistic was re-computed by averaging the absolute mean change difference per subject and summed over the whole group. This procedure was repeated with 9,999 permutations (with replacement for greater ease of computation). The result is shown in Figure 3.7B. In only 48 out of 10,000 permutations (incl. the original data set) was the summary statistic equal or greater than the observed value (sham: 26.65), which corresponds to a probability of $p = .005$. In contrast, the test statistics for the actual data when subtracting each active condition from all other conditions (Test statistic, HighInt: 12.80, LowInt: 15.92, LowCont: 18.51) fell into the 95% confidence interval of the null hypothesis distribution.

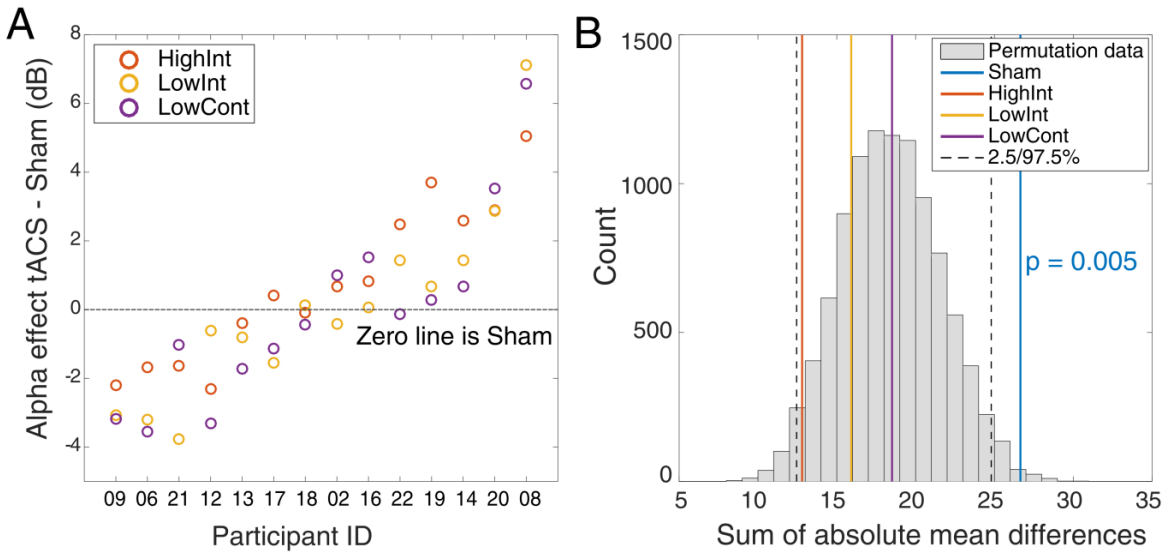

Figure 3.7: Idiosyncratic "alpha effects" to active tACS versus sham

A) Individual difference scores between changes in active tACS conditions and changes in the sham condition (in dB). Data points are sorted by mean α-effect in ascending order. Note that this order is arbitrary and has only been chosen to facilitate visualisation of the consistency in individual responses. Grey dotted zero line represents no difference to sham. *B)* Permutation analysis on the sum of absolute mean differences as a measure of consistent responses (for details see text). The histogram represents the expected distribution under the null hypothesis that the intra-individual α-effects are random. Coloured lines represent the test statistic calculated on the actual sample and for each condition. Vertical black dotted lines delimit the 95% confidence interval of the null hypothesis distribution.

This result appeared to indicate a qualitative difference for active versus sham protocols, despite a lack of finer nuances between active protocols. If the α-circuits were susceptible in general to near-dominant frequency stimulation independent of whether the stimulation frequency offset is slightly positive or negative, such a pattern would be plausible. However, as different participants reacted differentially with either α-enhancement or α-weakening, we would expect some property in either the particular stimulation parameters or individual characteristics to determine the direction of this effect. Therefore, we explored the association between a number of variables (pre-test power, stimulation intensity, day-to-day dominant α-frequency, relative mismatch between stimulation frequency and dominant frequency, peripheral sensations, baseline change during sham) and the α-effect (i.e., α-power change$_{\text{active tACS}}$ minus α-power change$_{\text{Sham}}$). The results are shown in Figures 3.8 - 3.12.

Alpha effect is weakly related to pre-test alpha power

People with generally higher levels of pre-test α-power tended to show α-weakening compared to sham, whereas those with relatively little power in the α-band tended to show enhancement (Figure 3.8). The Spearman rank

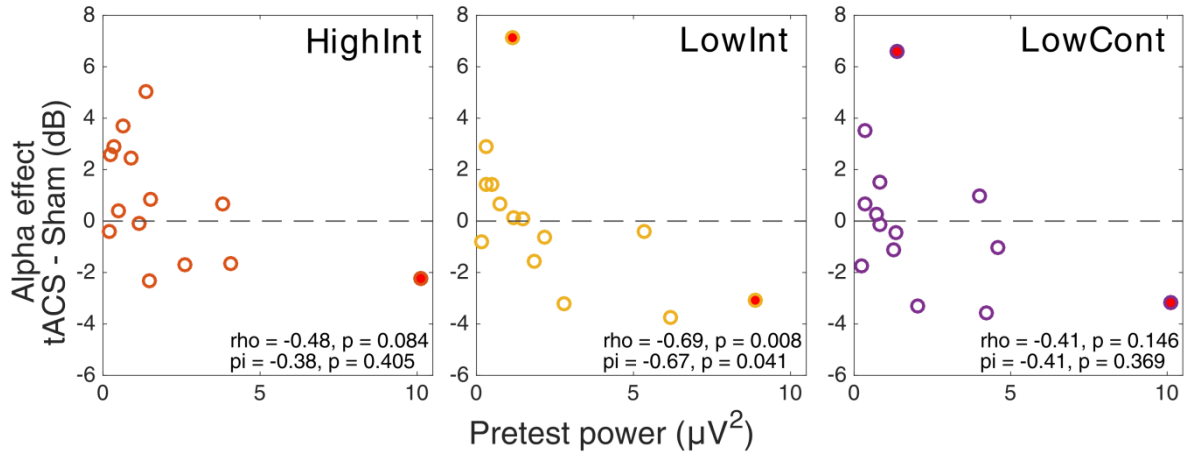

Figure 3.8: Pre-test alpha power versus alpha effect

Plots show a weak association between pre-test power in the individual α-band and the α-effect relative to sham in each active protocol. Note that as the intra-class correlation coefficient (ICC) is high for pre-test power and α-effect, the location of the data points representing each participant is similar across plots. Red filled circles are data points that have been identified as influential points (Shepherd's *pi*), i.e., omitting these data points from the analysis affects the strength of the statistical association.

correlation coefficient suggests a weak negative association between pre-test power and the tACS effect (HighInt: Spearman's *rho* = -.48, *p* = .084; LowInt: *rho* = -.69, *p* = .008; LowCont: *rho* = -.41, *p* = .146). The intra-class correlation coefficients (ICC; McGraw & Wong, 1996; type ICC(A,k) based on mixed effects model) for both pre-test measurements and tACS effect measurements across different sessions are high ($r_{ICC,\ pre\text{-}test}$ = .95; $r_{ICC,tACS\ effect}$ = .94), indicating that both variables were quite stable across sessions. In other words, participants showed similar pre-test α-activity but also a similar response to tACS compared to sham across sessions (and independent of protocol). Accordingly, there was no significant difference between pre-test measures across all four conditions (Friedman test, $X^2(3) = 1.63$, p = .65).

Alpha effect does not depend on tACS intensity

There was no evidence for a systematic relationship between current intensity and the tACS effect in any active condition (Spearman's *rho*: all $p > .68$; Figure 3.9A).

Alpha effect does not depend on dominant alpha frequency

There was no evidence for a systematic relationship between the dominant α-frequency on the day of testing and the tACS effect in any active condition (Spearman's *rho*: all $p > .27$; Figure 3.9B).

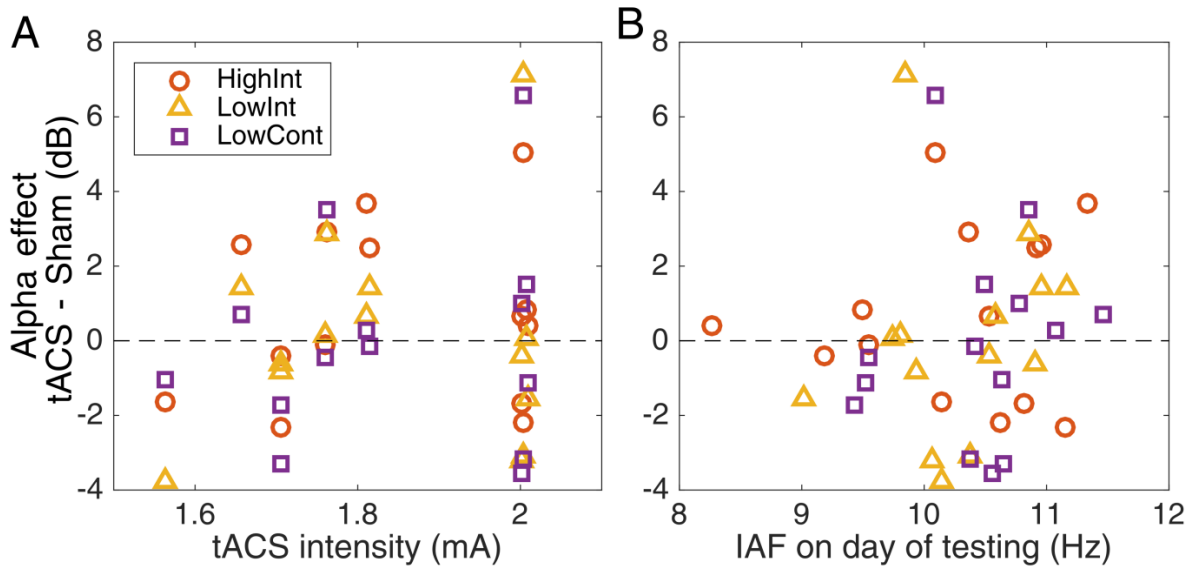

Figure 3.9: tACS intensity and dominant α-frequency versus alpha effect

There is no obvious association between *A)* tACS intensity (in mA) and *B)* variable dominant α-frequency (in Hz, estimated from the pre-test before each active session) and the α-effect relative to sham. As there were only weak associations in general, Shepherd's *pi* was not calculated.

Alpha effect does not depend on mismatch between stimulation frequency and dominant frequency

The distribution of estimated differences between the day-to-day IAF (defined as the mode of the bootstrapped peak frequencies of the average spectra at POz between 8 and 12 Hz, otherwise calculated as described above in the section *Individual alpha frequency*), and stimulation frequency (Figure 3.4) suggests a reasonable success in selecting frequencies just above or below the respective dominant frequency. However, at least in some cases the intended tACS-frequency may have been over – or underestimated, that is, may have either been too close to (data points within red boundaries) or too far removed from (data points on far left and right) IAF to be effective. In a couple of cases, the frequency in the low condition may have even been higher than the dominant frequency. Figure 3.10 shows the tACS effect as a function of the mismatch between stimulation frequency and the estimated dominant α-frequency on the day of testing. While there was a tendency towards a negative association between mismatch magnitude and α-effect in both low conditions that resembles the trend we found in Experiment 1 (Figure 2.6C in the previous chapter), we observed a similar pattern in the high condition. As a repeated measure with high intra-class correlation measures (see above), this

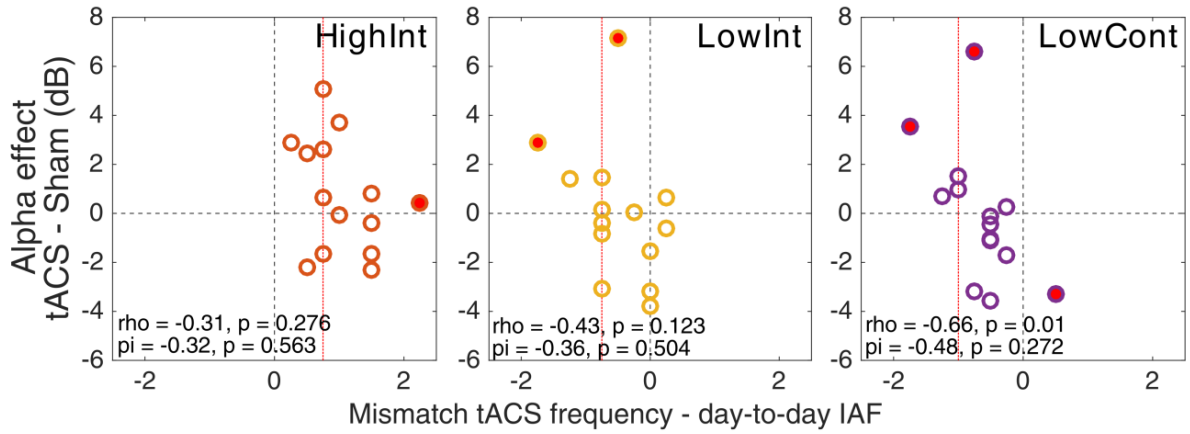

Figure 3.10: Alpha effect as a function of frequency mismatch

Mismatch refers to the difference between the variable dominant α frequency (in Hz, estimated from the pre-test before each active session) and the stimulation frequency of each active session. Red horizontal lines mark the intended stimulation frequency (i.e., IAF + 0.75 Hz and IAF - 0.75 Hz for high and low conditions, respectively). Vertical grey dotted line is day-to-day dominant α frequency. Rho = Spearman's *rho*. Pi = Shepherd's *pi*. Red filled circles are data points that have been identified as influential points, i.e., omitting these data points from the analysis affects the strength of the statistical association.

strongly suggests that the individual responses are not dependent on the specific frequency used, or the shift in frequency relative to the dominant α-frequency.

Alpha effect is only weakly associated with strength of peripheral sensations

The effect of tACS on α-power could be peripheral rather than cortically induced, for instance through a change in arousal. Different states of arousal have been associated with changes in spontaneous α-activity (e.g., Cantero, Atienza, & Salas, 2002; Makeig & Jung, 1995; Makeig, Jung, & Sejnowski, 2000; Shaw, 2003), and it is possible that intrusive and/or unpleasant peripheral sensations such as visual flicker or skin sensations may have resulted in systematic changes in individual arousal state (e.g., towards fatigue or agitation). In addition, work on transcorneal alternating current stimulation suggests that retinal activation mediates lasting elevation in α-power (Fedorov et al., 2011; Sabel et al., 2011; Schmidt et al., 2013; Sergeeva et al., 2015; Sergeeva, Fedorov, Henrich-Noack, & Sabel, 2012). In this case, the magnitude of the effect is likely independent of specific (similar) stimulation parameters but positively related to the intensity of the sensation. Figure 3.11 shows the individual α-effect as a function of the difference between VAS ratings in the active tACS protocols compared to sham. The difference in VAS scores for Itch and Discomfort were only weakly related to α-effects (Spearman's *rho*: all

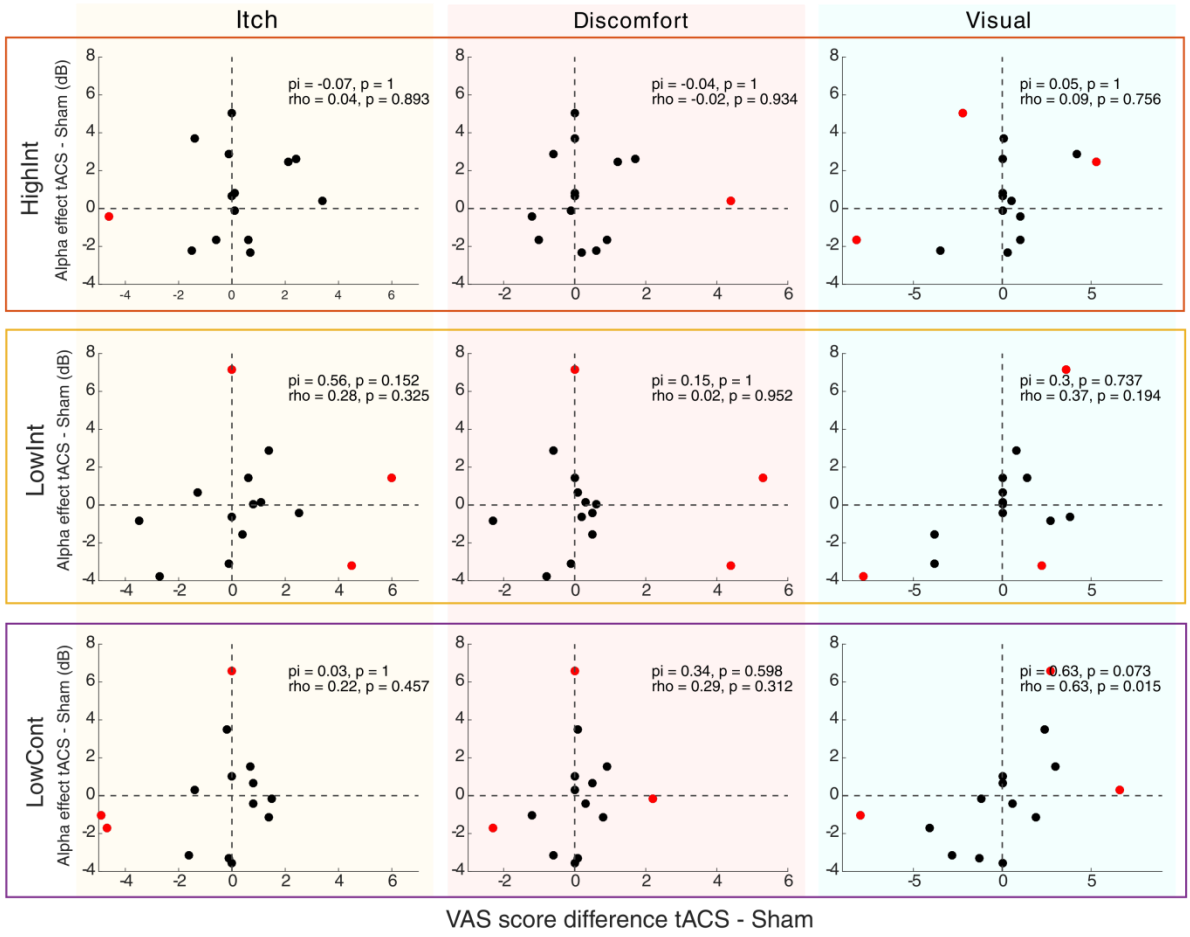

Figure 3.11*:* Alpha effect as a function of VAS score differences

VAS scores rating the peripheral sensations for sham were subtracted from each active condition. The vertical grey dotted line marks no difference in the respective sensation from sham. Rho = Spearman's *rho*. Pi = Shepherd's *pi*. Red filled circles are data points that have been identified as influential points, i.e., omitting these data points from the analysis affects the strength of the statistical association.

p > .32). For visual sensations in the low continuous condition, there was a positive association between difference score and α-effect (Spearman's *rho*: *p* = .015, all other *p* > .19), suggesting that participants who perceived more visual flicker/wobbling during active tACS than during sham tended to show α-enhancement, and participants who perceived less flicker/wobbling during active tACS tended to show α-weakening. This association becomes weaker when controlling for influential points (Shepherd's *pi* = .63, *p* = .073).

Alpha effect is dependent on sham baseline

As the estimate of the tACS effect (= $change_{activetACS}$ - $change_{Sham}$) is a derived measure, it is possible that differences in the baseline measure (here: α-power change in the sham condition) drive the differences in individual

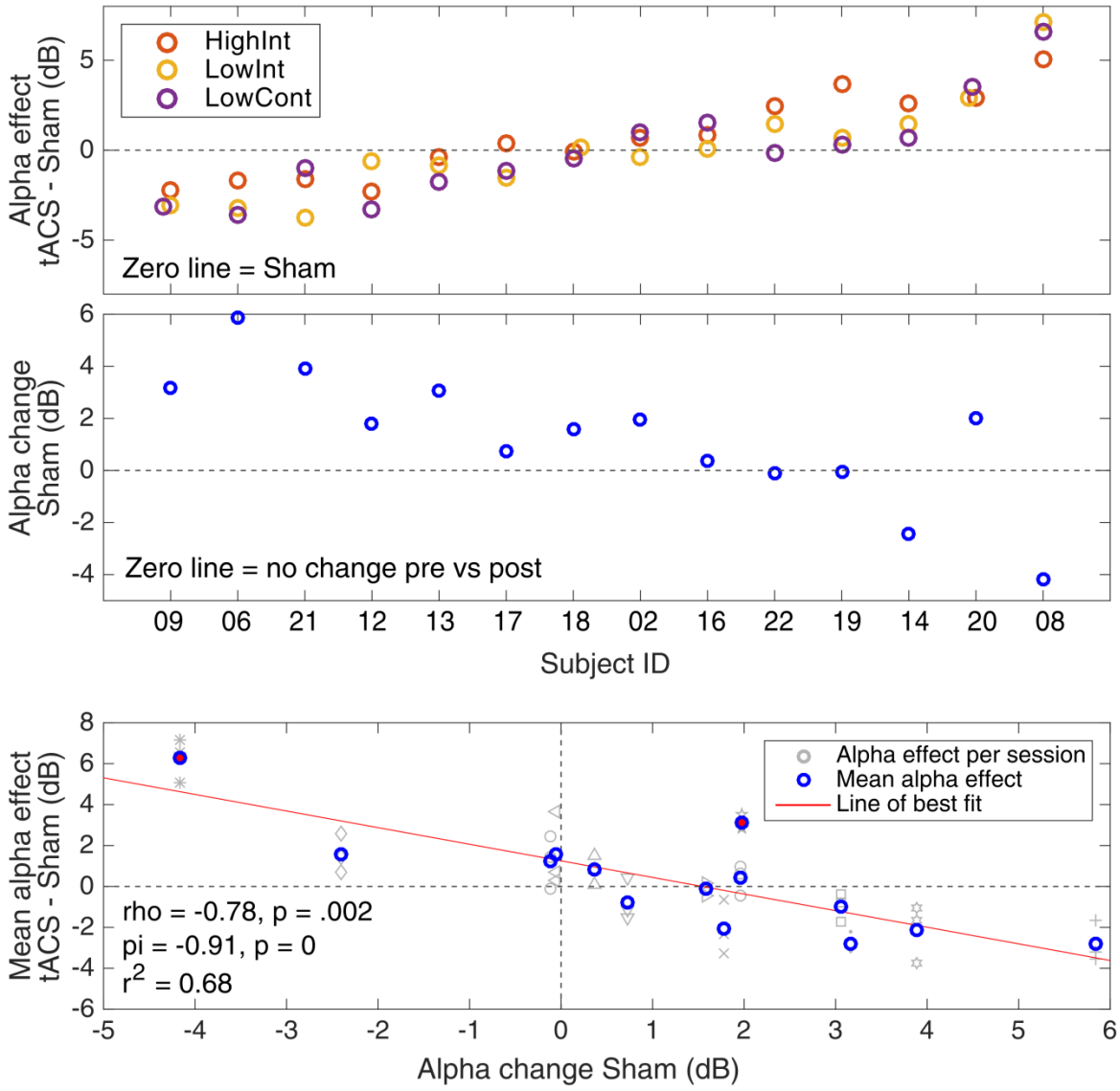

Figure 3.12: Association between alpha effect and sham baseline

These plots show the association between individual α-effect per condition and baseline (α-change during sham). *Top*: Same as Figure 3.7 (left) for ease of comparison. *Middle*: Alpha change during sham per participant, sorted by mean α-effect (i.e., same order as top panel). Note the difference in the meaning of the zero line. *Bottom*: Alpha effect as a function of baseline α-change during sham. Grey symbols represent individual conditions per participant; blue circles represent their mean α-effect. Red filled circles are influential points as identified by Shepherd's *pi* correlation.

α-effects. On closer inspection, it was confirmed that the α-effect (that is, the difference between the power changes in each active condition and sham) was strongly negatively correlated with the relative change in the sham condition (Spearman's *rho* = -.78, p =.002). As can be seen in Figure 3.12, the apparent order of participants according to their individual tACS response (top panel) is already predicted to a large extent by their α-power change in the sham condition (middle panel), and much of the variance can be explained by the differences in this baseline measure (r^2 = .68, based on Pearson's *r*, $\alpha\text{-change}_{Sham}$

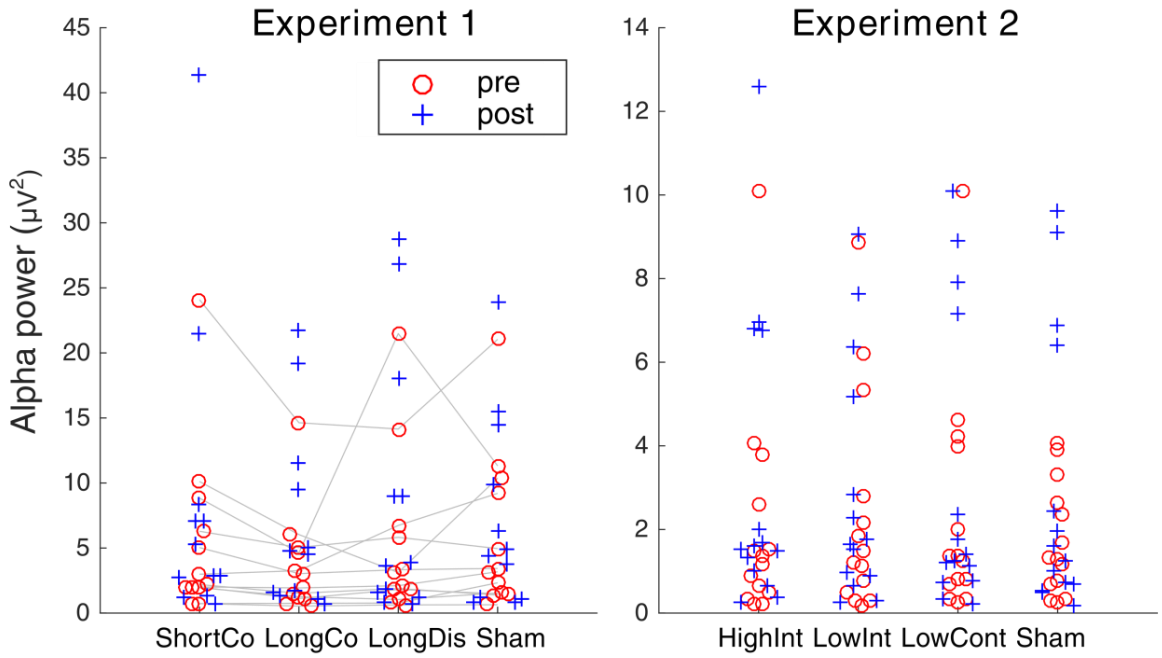

Figure 3.13: Alpha power differences between Experiments 1 and 2

Left: Alpha power in pre- and post-test in Experiment 1; *right*: Same for Experiment 2. Overall, α-power was higher/more variable in the first sample (note the difference in scale). Grey lines should help visualise the effect of tACS session in the pre-test within each individual, with lower power in LongCo compared to ShortCo and sham.

versus mean α-effect per subject, bottom panel). This strongly suggests that the index of ideosyncratic tACS effectivity (individually consistent changes across all tACS conditions when corrected for changes following sham stimulation) is driven by the common correction factor (change after sham), not the three tACS interventions.

Supplementary analysis: Experiment 1 revisited

The correlation of the change score with the baseline sham measure raises concerns about differences in baseline activity driving the effect found in Experiment 1. While this effect did not depend on change after sham as normalisation factor, post-test power was normalised by pre-test power and the dependent variable therefore potentially vulnerable to the same confound. Therefore, an additional analysis was run to compare absolute α-power between the different protocols in pre- and post-test, respectively. No differences between protocols were found either for pre- or post-test in Experiment 2 (both $p < .65$; Figure 3.13, right). In Experiment 1, while the Friedman test for post-test α-power was not significant ($X^2(3) = .90$, $p = .83$), there was a main effect of tACS protocol for the pre-test data ($X^2(3) = 9.8$, $p = .020$; Figure 3.13, left). Post

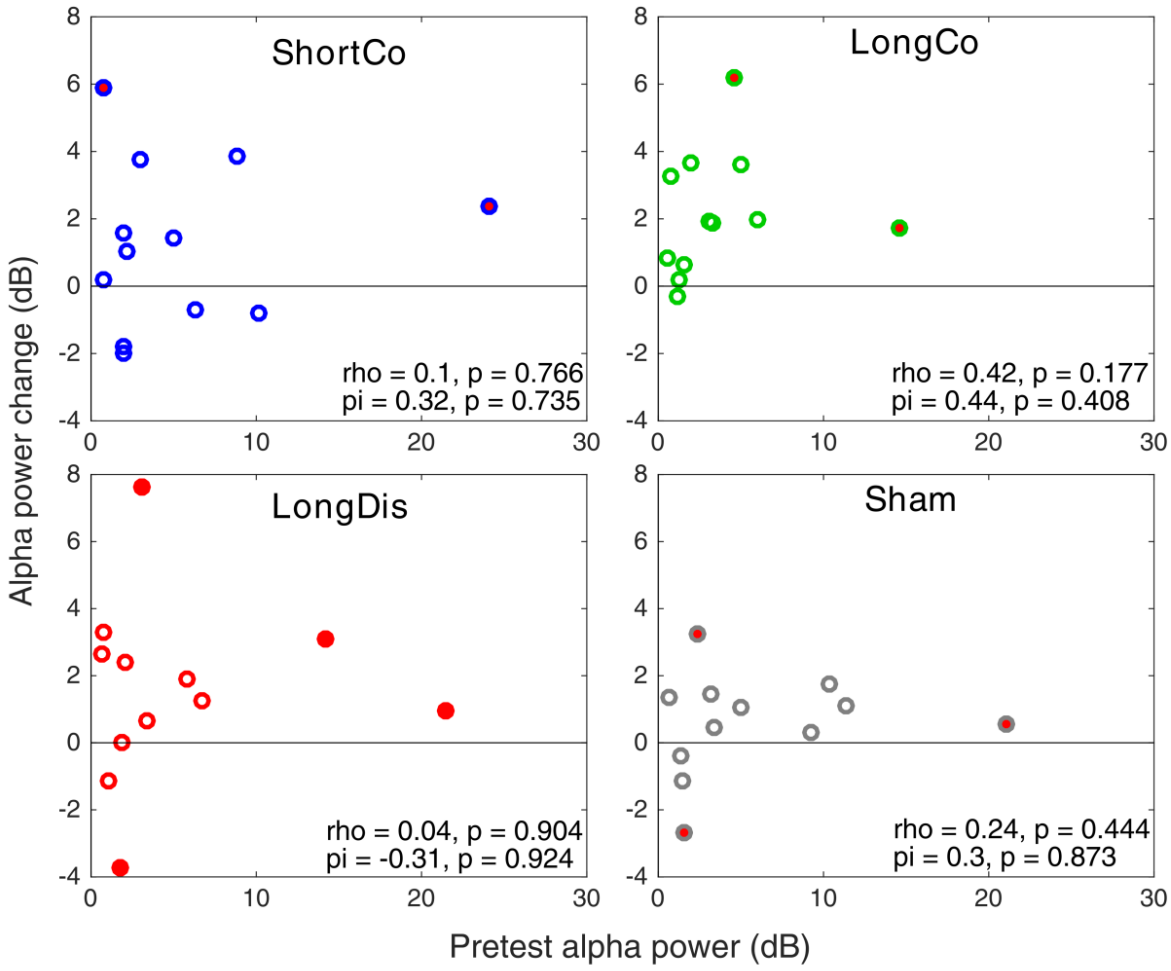

Figure 3.14: Pre-test α-power versus α-power change in Experiment 1

Low pre-test power does not predict a large α-increase. Red filled circles are influential points as identified by Shepherd's *pi* correlation.

hoc tests suggest that across participants, power was on average lower for the LongCo condition compared to either ShortCo (Z = 2.67, p = .008, uncorrected) or Sham (Z = 2.75, p = .006; all other comparisons p > .16). This result is still significant after Bonferroni correction. As there were no differences between protocols after post-test, this indicates that the tACS effect is mostly driven by pre-existing differences in baseline α-activity, mirroring the baseline confound observed in Experiment 2. However, α-power during pre-test did not predict the magnitude of α-power change in any condition (Figure 3.14).

Discussion

This study aimed to replicate the observation of α-enhancement after intermittent α -tACS, and to test the predictions of the spike timing-dependent plasticity model of tACS α-aftereffects. Fourteen healthy human participants were stimulated with intermittent tACS at a frequency either slightly higher or

lower than their IAF to probe whether the sign of the relative mismatch between IAF and tACS frequency determines whether α-activity is enhanced or suppressed. Disappointingly, the results from this experiment show no systematic effects of tACS on α-activity. The data not only fail to support the STDP model, but also do not replicate the α-enhancement after intermittent tACS observed in the previous experiment. Moreover, there was no α-enhancement after continuous α-tACS, contrary to several previous reports (Helfrich, Schneider, et al., 2014; Neuling et al., 2013; Zaehle et al., 2010). While it appeared at first that participants showed different but stereotypical responses to tACS independent of the specific protocol used, the strong correlation between these "responses" and the sham measurement they are derived from strongly suggests that the former are spurious.

Lack of alpha enhancement after intermittent tACS

A failure to enhance α-power with intermittent α-tACS has been reported by Strüber and colleagues (Strüber et al., 2015) after stimulation with trains of approximately one second (summing to a total of 2 x 5 min). These authors attribute their lack of effect to the short duration of the trains, which is in line with what we found for short (approximately 3 s) trains in Experiment 1. Apart from duration, there were a number of differences between their protocol and the 8 s protocols in Experiment 2, including a posterior midline tACS montage (Cz/Oz) and a five minute inter-stimulation break. More importantly, they directly compared the EEG in the intermittent silent periods before and after each tACS train. Experiment 1 has shown that within the time course of intermittent periods, an α-effect may have slowly been building up but was statistically discernible only after more than twenty minutes of prolonged intermittent stimulation. The finding of no change in Experiment 2, despite a train duration and overall stimulation time that were previously found to be effective, suggests (not unexpectedly) that train duration alone is insufficient to determine whether a protocol targeted at α-enhancement is effective.

Lack of alpha enhancement after continuous tACS

In contrast to previous studies (Neuling et al., 2013; Neuling, Rach, et al., 2012; Zaehle et al., 2010), the continuous protocol (at a long train duration of

15 min) also did not enhance α-power. Again, one might suspect differences in the specific stimulation parameters and montage (for instance, a Cz/Oz montage proved successful in Helfrich, Schneider, et al., 2014; Neuling et al., 2013), although the differences between this experiment and the pioneering paper of Zaehle and colleagues (2010) are small (per definition, as their study was the motivation for Experiment 1). Probably the most crucial difference is that Zaehle et al. employed a between-subject design, with one group of ten volunteers tested before and after α-tACS, and a separate sham group. Given the high inter-subject variability in α-power, it would be very informative to see individual spectral estimates within each group. Unfortunately, only group averages were supplied, and those will likely be driven by a few subjects with the highest α-peaks or the greatest changes. This makes it difficult to judge the robustness of their effect.

There are design problems in other studies that complicate the generalisability of their results. Neuling et al.'s first attempt at α-entrainment (2012) employed an oscillatory tDCS protocol, allowing for a possible contribution of tissue polarisation by the DC offset. However, as there was neither a sham group, nor a within subject sham condition, it is impossible to tell whether their observed α-enhancement was generic or somehow (whether through AC or DC currents) induced by electrical stimulation. While lasting α-power modulation was only secondary to their research question in this experiment, their 2013 study directly tested the effect of α-tACS on endogenous α-oscillations and included a sham control group. They found that when participants sat with their eyes open (that is, under conditions of relatively low α-activity), α-power was enhanced following tACS for at least an impressive 30 min. In contrast, in a parallel study where participants sat with their eyes closed (that is, with relatively high α-activity) no such enhancement was observed.

This study's results are also not unambiguous. Again, there are potential EEG differences in the individuals of the four groups tested. Moreover, in the eyes open experiment, eight (of thirty) volunteers were excluded because due to their higher z-score (> 1.65) in post-test power it was assumed that they had kept their eyes closed during the post-test. Based on our samples and the

literature (Shaw, 2003) it is safe to assume that high α-power can be present in some individuals even if their eyes are open, and that α-power measures are not necessarily normally distributed within groups, thus complicating the interpretation of what does and does not constitute an outlier. Rejecting participants based on high α-power alone therefore might increase the risk to draw false conclusions based on selective sampling. As an example recall that α-enhancement in Experiment 2 was somewhat more likely in participants with low pre-test α-power, whereas participants with the highest baseline α-power showed α-weakening compared to sham. The latter is more plausibly a case of regression to the mean, rather than some sort of trait-like responsiveness to stimulation, especially as pre-test power was not predictive of the magnitude of α-increase within a session. Yet, if we had chosen to reject data from individuals with high EEG α-activity, we may have concluded that tACS has a general enhancing effect. Undeniably, there are merits to the identification of outliers and subgroups; however, in small samples like in our and Neuling et al.'s study, with straight-forward research questions and an unknown sampling error or inter-subject variability, it is risky to over-interpret and to base analysis decisions on such differences. Nonetheless, this group has very successfully replicated their results using largely the same methods (Kasten, Dowsett, & Herrmann, 2016). The aftereffects in the latter study lasted up to 70 but less than 90 min.

What factors could explain the failure to replicate?

Why was the effect of tACS-induced α-enhancement not replicated? Despite a very similar experimental setup, there were some differences, the potential contribution of which will be discussed in turn.

Differences in design

First, a different EEG montage was used. Rather than recording frontal midline electrodes, additional electrodes were located over parietal cortex to provide converging information in the determination of the individual α-frequency. One possible concern is that the greater number of EEG electrodes in proximity to the tACS electrodes decreased the current density by effectively increasing the electrodes' surface area (assuming little resistance between the

tACS and EEG electrodes along the scalp), or resulted in increased shunting through the electrodes. The former does not seem likely, given the overall high impedance of the skin. The latter is also unlikely as the high input impedance of the EEG amplifier should not allow current flow out from the scalp (Pedro Miranda, who is expert on modelling electric fields induced by NIBS; personal communication). However, it is possible that the addition of Oz in particular, which is situated directly between the tACS electrodes, could have provided a shortcut and facilitated shunting of the current along the surface between the tACS electrodes. It should be noted, however, that other studies combining tES and EEG montages (e.g., Garside et al., 2015; Helfrich, Knepper, et al., 2014; Helfrich, Schneider, et al., 2014; Neuling et al., 2013; Pahor & Jaušovec, 2014; Voss et al., 2014; Vosskuhl et al., 2015), to name but a few) have reported stimulation effects despite this hypothetical problem, and integrated systems for combining EEG with electrical stimulation (e.g., STARSTIM, Neuroelectrics, Spain) allow explicitly for stimulating and sensing electrodes that can be allocated flexibly in a standard 10-10 system. Nonetheless, it should be established empirically and computationally that the proximity of passive, non-stimulation electrodes does not affect the current flow and/or density as to my knowledge this question has not yet been addressed.

Second, EEG was initially referenced to left mastoid to minimise contamination of the reference by cortical activity. Note that because this experiment looked only at offline effects, contamination by the sinusoidal tACS artefact is not a concern. If we assume that the topography of tACS-induced changes in α-activity was fairly homogenous over posterior scalp and the corresponding electrical potential changes equally picked up by the reference and the test electrode, these changes may have been obscured by subtraction of the electrical activity at the reference. Note that this assumption has to be constrained to specific α-activity, not to alpha per se, as most participants exhibited a more or less clear α-peak. This is a strong assumption that may not be likely to hold.

Third, the proximity of the test channel to the new reference Cz may have obscured the effect. The control analysis of Experiment 1, which shows that the effect remained stable after re-referencing, suggests that this is

implausible. However, to exclude these possibilities the protocols should be tested again using an identical montage as the available data do not allow for their immediate rejection.

Fourth, EEG data were recorded at a different resolution. The first experiment was recorded at 0.5 μV resolution to facilitate recordings during tACS by reducing the risk of signal saturation. As no online analysis was intended in this experiment, EEG was recorded at a resolution of 0.1 μV. However, a higher resolution should give a rather more specific estimate, not a different one. In sum, although there were differences in the experimental setup, it seems implausible that these differences are solely responsible for the lack of effect.

Pre-existing group differences in baseline alpha power

Notwithstanding the design limitations, if we accept the finding that under conditions of high alpha (that is, with eyes closed) tACS was ineffective - could the failure to enhance α-power in Experiment 2 depend on a different baseline α-state in this group compared to that in Experiment 1? It is conceivable that α-activity was already at a ceiling level for some participants in this sample. However, direct comparison between the independent samples of Experiments 1 and 2 shows that participants in the first group on average actually exhibited higher absolute α-power at baseline (Figure 3.13). Of course there is no a priori reason to believe that the strength of α-activity can be quantitatively meaningfully compared between individuals. Looking at the differences between conditions within pre-tests and post-tests, however, we observe: Firstly, that there are no post-test differences between conditions in either group; secondly; that the second group shows no pre-test differences; and thirdly, that in the first group there is a significant pre-test main effect of tACS protocol, driven by lower α-power in the LongCo condition compared to both ShortCo and Sham. Recall that LongCo was the most effective condition for α-enhancement relative to sham. Indeed ten out of twelve participants showed lower α-power during the pre-test of the LongCo session, compared to that of the sham session.

As there was no systematic difference in post-test power between protocols, and moreover no significant increase from pre- to post-test following sham, one could assume that during the sham session, most participants already began with a saturated level of α-activity, leaving no room above to allow further increase. In contrast, during the LongCo session participants did not start at ceiling α-levels and could increase towards their saturation level (this assuming that there is a maximum sustainable level of α-activity). The change in power would solely be driven by baseline state. However, in this simplified situation, we would expect α-power during pre-test and the amount of change from pre- to post-test to be strongly anti-correlated within a given session (with little increase at high levels of pre-test power and large increase for low levels of pre-test power). These expectations were not really met (Figure 3.14). Moreover, no such pre-test difference was present between the other effective protocol, LongDis, and Sham. Therefore, the origin of the effect remains somewhat inconclusive, and while it is plausible that pre-test differences contribute to the observed differences in α-power change in Experiment 1 they may not tell the whole story. However, the ambiguity of these results underlines the difficulty of making sound statistical inferences based on small sample sizes, and together with the lack of effect in Experiment 2 constitute a warning that the effects of α-tACS on α-activity are weak and difficult to reproduce at best.

Ostensible individual differences

The literature on individual variability in NIBS response (reviewed in Ziemann & Siebner, 2015) suggests the possibility that tACS was effective on an individual level but that participant-specific responses were obscured at the group level. To account for individual response patterns, we tried to identify variables in Experiment 2 that predicted the specific outcome of tACS versus sham. First, we found that the specific stimulation parameters that would be expected to determine current strength and entrainment efficacy (intensity, frequency relative to the endogenous oscillation) did not show any relationship to the tACS effect. This is consistent with Experiment 1 where frequency mismatch did not produce an entrainment-like pattern and would support a plastic mechanism that is relatively frequency-independent (or at least forgiving within a yet-to-be-determined range). Secondly, of the peripheral sensations ratings, only subjective reports of unusual visual percepts showed an

association, and only in the continuous condition. This would not rule out a contribution of unintentional side-effects but provides no strong evidence for such a contribution either. Thirdly, state variables such as the individual α-frequency and pre-test power would be expected to modulate the efficacy of tACS. Only pre-test power was marginally predictive of α-power enhancement relative to sham, yet this association (just as all others) is called into question by the strong correlation between the change scores and the baseline sham measure. The bottom line is that when analysing individual response patterns it is easy to overlook confounding variables and draw erroneous conclusions, especially when using aggregate measures such as that describing the tACS effect in question.

Data analytical considerations

One could argue that because of the small sample, there was a lack of statistical power to find a subtle effect of tACS. However, any reasonable estimate of effect size would be based on the results of the first experiment, in particular for the intermittent protocols; as there were fewer participants in Experiment 1 than in Experiment 2, one would expect that in the latter it should have been *easier* to detect a robust stimulation effect (i.e., an effect that is common to the majority of participants). This was not the case, suggesting lack of power was not at the root of the failed replication.

Naturally, we have to ask whether the results of Experiment 2 constitute a type II error – failure to reject the null hypothesis that tACS is no more effective than sham in enhancing α-power – or whether the results of Experiment 1 rather represent a case of type I error. Generally, as the sample size increases, the likelihood of obtaining a more extreme ratio between two categorical outcomes by chance decreases (e.g., assuming the distribution is uniform, for a sample size of four a ratio of 3:1 between two categories is more likely than for a sample size of 400). In a smaller sample, it is more likely that a majority of data points fall into any one category by chance (here: successes, i.e., tACS > sham, versus failures, tACS < sham). Assuming four equal conditions, each participant has a 25% chance of ending up with sham as the least effective condition; the more independent participants, the less likely it becomes that a majority will cluster in one condition by chance. The statistical test in

Experiment 1 suggests that the observed ranking is unlikely due to chance, and intuitively it is tempting to believe in an effect when all three active conditions have more successes than the control condition. However, intuitions – and statistical tests - can fail, and a type I error is possible.

One possible problem obscuring a null effect is the ambiguity of difference scores. Problems with the reliability and validity of difference scores have been discussed elsewhere (J. R. Edwards, 1994; Tisak & Smith, 1994). In our specific case, the ranking procedure used in the nonparametric statistical tests counted both actual increases in α-power (i.e., a greater increase from pre- to post-test in a condition, relative to another) as well as lesser *de*crease over the course of a session as "more effective" (i.e., successes). While based on the idea that α-tACS stabilises the underlying α-circuitry this could be interpreted as preventing "α-disintegration", this notion remains speculative; importantly, it lumps together what may not belong together, and skew the conclusion of the test into an unwarranted direction. This problem is exaggerated when the differences score between active and sham protocols are considered: A positive difference score (or "tACS effect") can mean either a greater increase, an increase compared to a decrease (which would both qualify as α-enhancement), or a smaller decrease after tACS relative to sham (less α-disintegration). The opposite applies to a negative tACS effect. However, when controlling for this possibility in Experiment 1, only one person per active condition that was categorised as success (i.e., showed α-enhancement compared to sham) falls into the speculative "prevented α-disintegration" category. These data belong to two participants who responded atypically (for that sample) with a strong α-decrease in most conditions including sham. This also means that in line with the original conclusion (i.e., in support of a real effect), the majority of participants in Experiment 1 did respond with a relative α-increase following active tACS compared to sham.

The problem of difference scores is related to that of finding an appropriate and reliable baseline. In light of the inter-individual *and* intra-individual variability of α-activity, and in order to avoid interaction analysis which is not possible with the Friedman test, we decided to normalise the data to a person's pre-test activity on any given day. Implicit in this decision are,

however, two assumptions: first, that the reactivity towards tACS is independent on the baseline level of α-activity, an assumption that is called into question by findings of state-dependency of tES effects (Benwell et al., 2015; Feurra et al., 2013; Feurra, Bianco, et al., 2011; Marshall et al., 2011; Neuling et al., 2013; Ruhnau, Neuling, et al., 2016). Essentially, we do not know whether α-enhancement might be additive or multiplicative, follows a U-shape or any other non-linear function, or whether the tACS effect is bistable and can transition into enhancement or weakening at given levels of α-activity. Second, that each power measurement is a reliable estimate of α-activity. In contrast to tES studies using motor evoked potentials (MEPs; Antal et al., 2008; Nitsche & Paulus, 2000; Schutter & Hortensius, 2011), where the intensity of a TMS pulse can be adjusted to produce MEPs of uniform amplitude, no method exists by which the level of α-activity can be normalised. As α-activity reflects an ongoing process that unfolds and changes over time, rather than a temporally restricted impulse response, it is by no means trivial that such a method (to produce "unity α-power") could be developed in principle. The lack of appropriate baseline conditions makes any assessment across different conditions and test days a difficult undertaking, and a lot more research into the causes and extent of intra-individual α-variability may be required before reliable comparisons can be made.

Finally, the dependent variable of mean power in the individual α-band may not be a robust indicator of α-activity. This variable has properties that cast doubt on its usefulness as a descriptive statistic. First, mean α-power is not uniformly distributed across trials. For most participants, the distribution is highly positively skewed, with the bulk of data points in the low power range and only a few high power trials. This is not surprising, given the discontinuous, burst-like nature of α-dynamics. However, it means that individual estimates of overall α-activity are biased towards a few trials of high activity and may not be representative of the "average" brain state. As such, although the power distribution of pre- and post-tests may be very similar, only a couple of outliers can lead to the conclusion that one has higher power than the other. With subsequent analysis steps on such unstable estimates, the error is perpetuated and lead to inconsistent results. Indeed, if the median (rather than the mean) across trials is used, the main effect in Experiment 1 disappears ($X^2(3) = 2.7$,

p = .44). While other groups (Neuling et al., 2013; Zaehle et al., 2010) found good effects using mean α-power, this is a potential caveat, and it should be considered carefully whether a small number of additional α-bursts or bursts with higher power qualify as genuine neural network change.

Conclusion

The null result of this second experiment does not allow us to speculate about the implications for the spike timing-dependent plasticity hypothesis of tACS aftereffects. In addition, the failure to replicate the α-aftereffect in an independent sample using very similar protocols raises questions as to the robustness of the original findings and the validity of their interpretation. While some of the experimental parameters were different, and the consistent enhancement relative to sham in Experiment 1 cannot easily be discussed away, this result underlines the need for replication studies, large sample sizes, and careful statistical procedures in order to identify genuine aftereffects on α-oscillations, and the appropriate conditions under which they occur.

Chapter 4. No evidence for a role of alpha entrainment in visuospatial bias induction when lateralised α-tACS is applied to the right occipito-parietal cortex (Experiment 3)

Lasting effects on neural activity as those tested in the previous experiments are not the only promising feature of tACS. tACS is also increasingly used in studies of cognition and perception as a tool to stimulate - or emulate - oscillatory brain activity while volunteers are actively engaged in tasks that are thought to hinge on specific brain rhythms. Traditionally, the function of neural oscillations has been deduced from task-related changes in EEG or MEG signals and their correlation with behavioural variables. The appeal of tACS lies in the similarity of the applied waveform to the periodic nature of these oscillations, which promises to allow causal investigation of the functional relevance of specific frequency bands and their interactions.

Due to the difficulties in obtaining an artefact-free signal, not many EEG/MEG studies have looked directly at changes in neural synchrony during (i.e., online to) tACS, but as reviewed in the introduction, evidence is slowly accumulating that ongoing tACS can entrain network activity at stimulation frequency at least in networks with a similar intrinsic frequency (e.g., Ali et al., 2013; Fröhlich & McCormick, 2010; Helfrich, Schneider, et al., 2014; Neuling, Rach, et al., 2012; Ozen et al., 2010; Riecke, Formisano, et al., 2015), with potentially measurable behavioural consequences. For instance, oscillatory stimulation has been shown to modulate auditory (Neuling, Rach, et al., 2012; Riecke, Formisano, et al., 2015; Riecke, Sack, et al., 2015) and visual perception (Helfrich, Schneider, et al., 2014) in a phase-dependent manner.

A critical design aspect for tACS experiments aiming at behavioural changes through modulation of oscillatory activity is a well-documented association between the targeted behaviour and regional rhythmic activity. One of the best-studied and frequently replicated oscillatory patterns is the lateralisation of α-power over occipito-parietal cortices associated with covert visuospatial attention. When an individual covertly directs spatial attention to the left or right visual hemifield (that is, without moving the eyes towards the attended location) α-power is typically found to increase over the visual cortex

in the ("ignoring") hemisphere ipsilateral to the attended location (which receives input from the contralateral irrelevant hemifield), relative to the contralateral ("attending") hemisphere (Rihs et al., 2007; Sauseng et al., 2005; Thut et al., 2006; Vossen, Ross, Jongen, Ruiter, & Smulders, 2016). This α-increase may be paired with an increase in gamma (γ-)activity in the contralateral hemisphere (Doesburg, Roggeveen, Kitajo, & Ward, 2008; Fries, Reynolds, Rorie, & Desimone, 2001). Recall that according to the gating by inhibition hypothesis (Jensen & Mazaheri, 2010), reduced α-power giving rise to enhanced γ-power allows enhanced local processing in the attending hemisphere, while alpha serves as an inhibitor of irrelevant information in the non-attending hemisphere (Bonnefond & Jensen, 2013).

In addition to power, the phase of ongoing alpha is influential in determining visual and auditory detection probability (Mathewson et al., 2011; VanRullen et al., 2011). Importantly, it has been shown also that tES with 10 Hz modulation also imposes a phase-dependent pattern on auditory perception (Neuling, Rach, et al., 2012), although such phase-dependence has also been observed with 4 Hz stimulation (Riecke, Formisano, et al., 2015; Riecke, Sack, et al., 2015).

Finally, in line with the role of posterior parietal cortex (PPC) in the allocation of spatial attention (Corbetta & Shulman, 2002), interventional studies that applied repetitive TMS to (especially right) PPC, in particular the intraparietal sulcus (IPS) have observed impaired detection of peri-liminal contralateral visual targets (Capotosto, Babiloni, Romani, & Corbetta, 2009, 2012; Hilgetag, Théoret, & Pascual-Leone, 2001; Romei, Gross, & Thut, 2010). This impairment has been related to the disruption of event-related desynchronisation and lateralisation of anticipatory α-activity while preparing for an expected stimulus (Capotosto et al., 2009, 2012). Romei and colleagues (Romei et al., 2010) found this impairment to be specific to stimulation at 10 Hz, compared to 5 and 20 Hz and sham, when the pulse train was applied immediately before the onset of a dot probe in a visual detection task, suggesting α-entrainment by rhythmic TMS.

Because the association of the alpha rhythm with anticipatory spatial attention is well established, it seems an ideal target to test claims of

frequency-specific tACS modulation. Accordingly, Brignani and co-workers (Brignani et al., 2013) have previously investigated whether tACS applied over either left or right occipito-parietal cortex can mimic the suggested role of α-lateralisation in form of location- and frequency-specific effects on visual processing of low contrast Gabor patches. Participants were asked to respond, firstly, whether they had seen a stimulus on a display with two placeholders and, secondly, which of two orientations that Gabor stimulus had contained. They found that detection, but not discrimination, was impaired by tACS at both 6 and 10 Hz, but not 25 Hz, compared to baseline and sham. More specifically, this impairment was manifest in failure to improve performance on the detection of Gabor patches across two successive sessions, in the authors' words "a suppression of learning". This effect was not dependent on target location, hence not confirming artificial, topographically specific attentional bias.

Brignani and co-workers listed a number of shortcomings that make the results difficult to interpret. First, tACS was applied in a between-group design. Moreover, contrast was fixed across all participants, rather than individually adjusted. Large inter-individual differences in performance such as ceiling effects in some participants may have masked subtle effects. Second, α-tACS was applied at a fixed frequency of 10 Hz, rather than at individual alpha frequency. Synchronization of an oscillatory network would be expected to occur specifically when the stimulation frequency matches the intrinsic frequency (Pikovsky et al., 2001). This applies especially when the synchronising force is weak, as is the case for the weak electrical current induced by tACS at the cortical level. In addition, people with a low intrinsic alpha peak frequency might actually respond equally well to 6 Hz than 10 Hz stimulation, potentially explaining the rather broadband response. Third, the montage used consisted of either PO7 or PO8 as target electrode, and the return electrode centered over the vertex (Cz). As in tES methods both electrodes contribute to the overall effect, it is possible that midline stimulation prevented proper lateralisation, explaining lack of hemifield specificity. Finally, tACS was applied in three continuous blocks of 5 min. With prolonged stimulation, homeostatic mechanisms may actively counteract the effects of stimulation, rendering it ineffective (Karabanov et al., 2015).

The current pilot experiment attempted to induce a visuospatial bias through lateralised α-tACS by addressing these shortcomings directly. If tACS can entrain α-oscillations related to covert spatial attention, an optimised experimental design should be able to affect visual performance in a hemifield-specific manner. To assess frequency-specificity, tACS was also applied at 40 Hz (in the gamma range).

The following design features addressed directly the shortcomings in Brignani et al. (2013): First, the study used a within-subject design and titrated stimulus sizes to account for inter-individual variability; second, tACS was applied at individual α-frequency to maximise the chance of α-entrainment; third, the electrode montage (including the reference) was lateralised to the right hemisphere over posterior parietal and occipital cortices; fourth, an event-related intermittent design was applied to avoid order effects and homeostatic plasticity. With these features, we aimed to improve both the effectiveness of tACS and the interpretability of the outcome. In addition, by recording the tACS-phase in which the visual target was presented we could explore phase-dependency of visual detection as additional evidence for or against α-entrainment.

We hypothesised that if alpha-tACS induces or emulates α-oscillations (via entrainment or network resonance) that are relevant to spatial attention by suppressing information with topographic specificity, right-hemispheric α-tACS should lead to worse detection of targets in the left hemifield (contralateral to the "inhibited" hemisphere) and/or improved detection of targets in the right hemifield (contralateral to "attending" hemisphere) (Figure 4.1A).

In contrast, given the proposed role of γ-oscillations in enhanced local processing for visual information at attended locations, γ-tACS should have no effect at all or result in the opposite effect, i.e., improved detection in the left hemifield (Figure 4.1B).

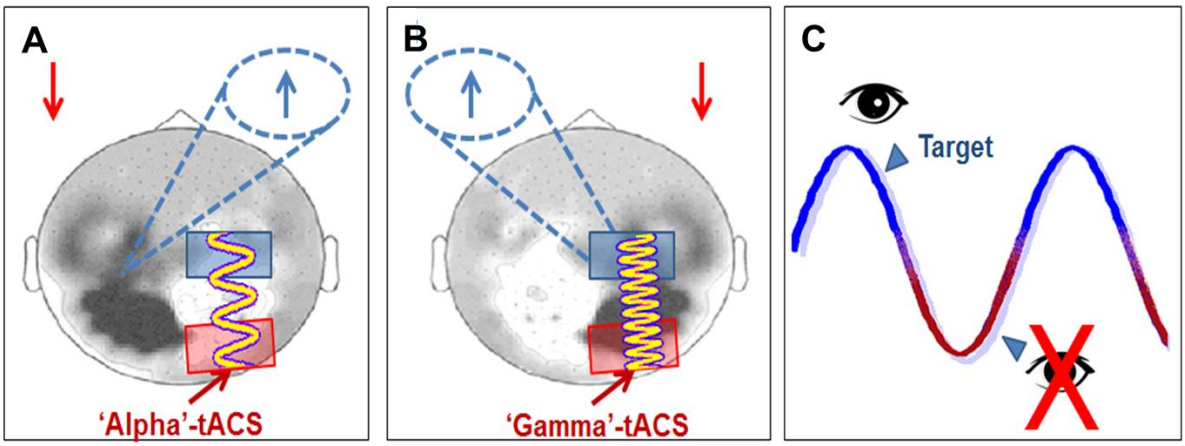

Figure 4.1: Graphical representation of the three main hypotheses
For details please refer to text.

Further, previous observations of α-phase dependence of visual processing suggests that detection performance should correlate with the tACS phase in which targets are presented, i.e., the phases of trials with correct and incorrect responses should have different distributions (Figure 4.1C).

Methods

Participants

Twenty healthy participants were recruited from students and the local subject database (9 male, age range 19 - 28 years, *M* = 22.7, *SD* = 2.2). All except one were right-handed. All volunteers gave written informed consent and received monetary compensation £9/hour for their participation. The study was approved by the local ethics committee of the College of Science and Engineering, University of Glasgow. No participants reported a history of neurological/psychiatric disorders or any other contraindication to tACS (current use of psychoactive medication/drugs, metal implants, pregnancy; see Appendix A for screening questions). For participant demographics and stimulation parameters see Table 4.1.

Table 4.1: Subject demographics and experimental parameters

IAF = individual alpha frequency. mA/pp = milliampere peak to peak.

ID	*Sex*	*Age*	*Handed-ness*	*IAF (Hz)*	*Intensity (mA/pp)*	*Dot size (unilateral)*	*Dot size (bilateral)*
1	f	23	**L**	11.50	1.50	9	9
2	m	28	R	11.50	1.00	9	9
3	f	25	R	10.25	1.10	9	9
4	m	24	R	10.00	1.15	9	9
5	f	22	R	11.25	1.00	9	9
6	m	23	R	12.25	1.70	9	9
7	f	25	R	10.25	1.50	9	9
8	f	23	R	10.75	0.90	**6**	**9**
9	f	25	R	12.50	1.80	4	4
10	f	23	R	12.25	1.80	**9**	**4**
11	f	19	R	12.25	1.10	**6**	**9**
12	m	20	R	9.00	1.15	6	6
13	m	20	R	11.00	1.20	6	6
14	m	21	R	10.00	1.10	6	6
15	m	23	R	12.25	1.70	4	4
16	m	23	R	11.00	1.80	6	6
17	f	22	R	12.50	1.40	4	4
18	m	23	R	11.25	2.00	4	4
19	f	19	R	10.50	1.70	16	16
20	f	22	R	11.00	1.30	4	4

Tasks and visual stimulation

Participants performed a dot titration task and an experimental dot detection task (Figure 4.2). The tasks were similar to the task described in Romei et al. (2010) and presented using E-Prime 2.0 (Psychology Software Tools, Sharpsburg, PA). A fixation cross and two placeholder squares were continuously displayed on a light grey background on a CRT monitor (display size 16 x 12 inch, resolution 1280 x 1024 pixel, refresh rate 85 Hz) . The fixation cross (width 0.7 degree of visual angle) was presented in the upper part of the screen, and the two placeholders (width 2.0 degree) were presented at 4.1/3.7 degree eccentricity (horizontal/vertical) in the lower left and right visual fields. A trial was initiated by an alerting cue, with the fixation cross turning from black to grey for 600 ms.

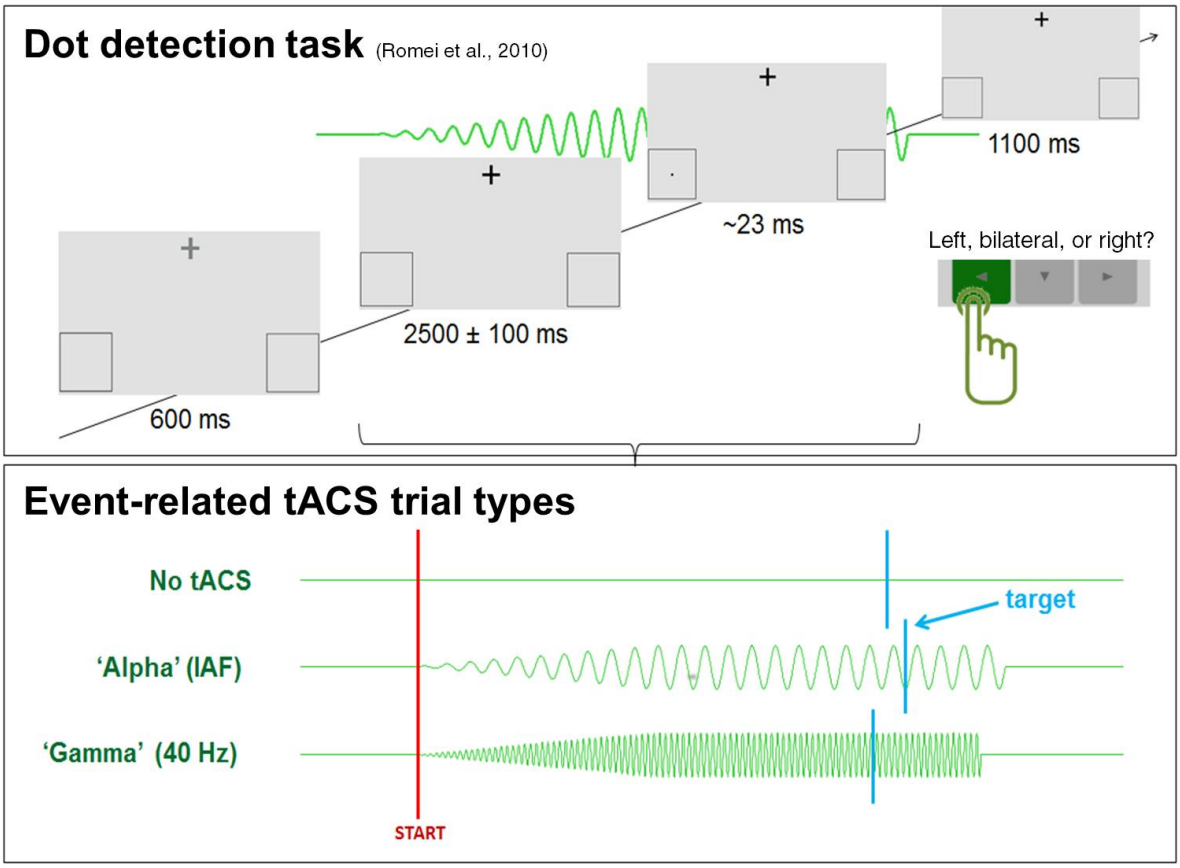

Figure 4.2: Experimental design

Top: Schematic of a typical trial of the dot detection task. In this example a target is presented in the left hemifield. *Bottom:* Examples of the different tACS trial types. In active stimulation trials, the electric current was initiated at either individual alpha frequency (IAF) or at 40 Hz, ramped up over 1 s, and remained at individually determined intensity until 300 ms after target onset (blue vertical line) or maximally 2 s during catch trials. In No tACS trials, only the target was presented.

In the *dot titration task*, after a variable interval between 600 and 1000 ms a "dot" (i.e., small black rectangles) appeared in one of eight different sizes (1 x 1, 1 x 2, 2 x 2, 2 x 3, 3 x 3, 3 x 4, 4 x 4, or 4 x 5 pixels) for around 23 ms (two refresh rates) in either of the placeholder boxes (27 trials per size and location plus 27 catch trials without stimulus, i.e., 459 trials in total presented in random order). Participants had to indicate within 1300 ms by pressing either the left or right arrow key on a keyboard where they had detected a dot. They were explicitly instructed to follow their gut feeling but not to make blind guesses as catch trials would be present. The dot size with accuracy closest to but still above chance level (47%, accounting for catch trials) was chosen as perithreshold stimulus for the experimental task, aiming at an overall performance level between 60 and 80% in the experimental dot detection task to leave room both for tACS-induced improvement and deterioration.

The *experimental dot detection task* (Figure 4.2, top) only differed in the following aspects: The alerting cue was followed by an interval of between 2400 and 2600 ms before the target presentation. Only one (peri-threshold) dot size was presented in all trials. In contrast to the titration task, dots could appear either left, right, or bilaterally. On tACS trials, tACS was initiated with the offset of the alerting cue and remained on until 300 ms after target disappearance or for maximally 3 s in catch trials. Participants had to indicate the target location by pressing the left, right, or down key (for bilateral trials) within 1100 ms. There were sixty target trials per tACS condition (Alpha, Gamma, Sham; see tACS) and location as well as thirty catch trials, i.e., 570 trials in total) presented in random order.

Procedure

Each participant underwent two sessions. In the first session, they received information on the study and filled in screening forms and informed consent. Then they were seated in front of a computer monitor with their head resting on a chin rest at a viewing distance of around 57 cm. First they performed the dot titration task (including ten practice trials). Then the EEG was prepared, followed by recording of resting EEGs with eyes open (4 min, while maintaining fixation on a white fixation cross on a dark-grey background presented on the computer monitor) and closed (3 min). Finally, EEG was recorded while participants performed a training session of the experimental task with the dot size individually adjusted based on the titration task results (approximately 5 min). The first session took approximately one hour.

The second session started with the preparation of the tACS electrodes and adjustment of each participant's current intensity level below phosphene- and discomfort threshold. To this end an initial train was given for eight seconds at 0.9 mA at the person's IAF established in the previous session (see section *EEG and estimation of individual alpha frequency*). The current intensity was then increased until either the participant reported unusual visual disturbances such as phosphenes or wobbling of the visual field, or until the participant expressed discomfort. Note that with this montage, participants generally had much lower sensation thresholds compared to the one used in experiments 1 and 2. To allow a few minutes for the electrode gel to settle and the impedance to drop,

participants performed another brief practice of the experimental task. If their performance had improved or deteriorated significantly from the previous day, a smaller or greater dot size was subsequently chosen. Then the experimental task (including tACS) was administered. Impedance was checked during regular self-timed breaks after which ratings of visual flicker/phosphenes, skin sensations, and discomfort (seven-point scale from "not at all" to "very strongly") were obtained (see Appendix C for rating questions).

EEG recording and estimation of individual alpha frequency

Recordings were obtained from six scalp locations (approximately at positions O1/2, P7/8, AF3/4 according to the 10/20 system with CMS/DRL reference over P3/4) using an Emotiv wireless headset (Emotiv, San Francisco, USA) at a sampling rate of 128 Hz, a bandwidth of 0.2 - 45 Hz, and a resolution of .0.51 µV. EEG was recorded at rest with eyes open (4 min), eyes closed (3 min), and during practice of the experimental task ("on task"). To obtain task-related IAF, on-task EEG was detrended, demeaned, and segmented into consecutive 1 s epochs. Epochs contaminated with eyeblinks or other artefacts were removed. Data were then re-referenced to the average of AF3/4 to emphasise posterior alpha activity. Single epoch spectra were obtained by Fast Fourier Transform (2 - 18 Hz with 0.25 Hz frequency resolution, Hanning window, 4 s zero-padding). The stimulation frequency was determined by visual inspection of single trial and average power spectra at the remaining electrodes by identifying the peak in the alpha range between 7 and 13 Hz (see Table 4.1). Stimulation frequencies ranged from 9 - 12.5 Hz (*M* = 11.16, *SD* = .98).

tACS

tACS was administered through a battery-driven constant current stimulator (DC Stimulator Plus, NeuroConn, Ilmenau, Germany). Electrodes were 5 x 7 cm^2 rubber rectangles with a layer of Ten 20 electrode gel which were placed above 10-10 system locations C2/4 and O2/PO8 (with left edges aligned to midline), respectively. Electrodes were additionally fixed with rubber bands. In this setup, the current at one electrode was in anti-phase to the current at the other electrode. tACS intensity was adjusted individually below phosphene- and discomfort threshold but held constant across conditions for each

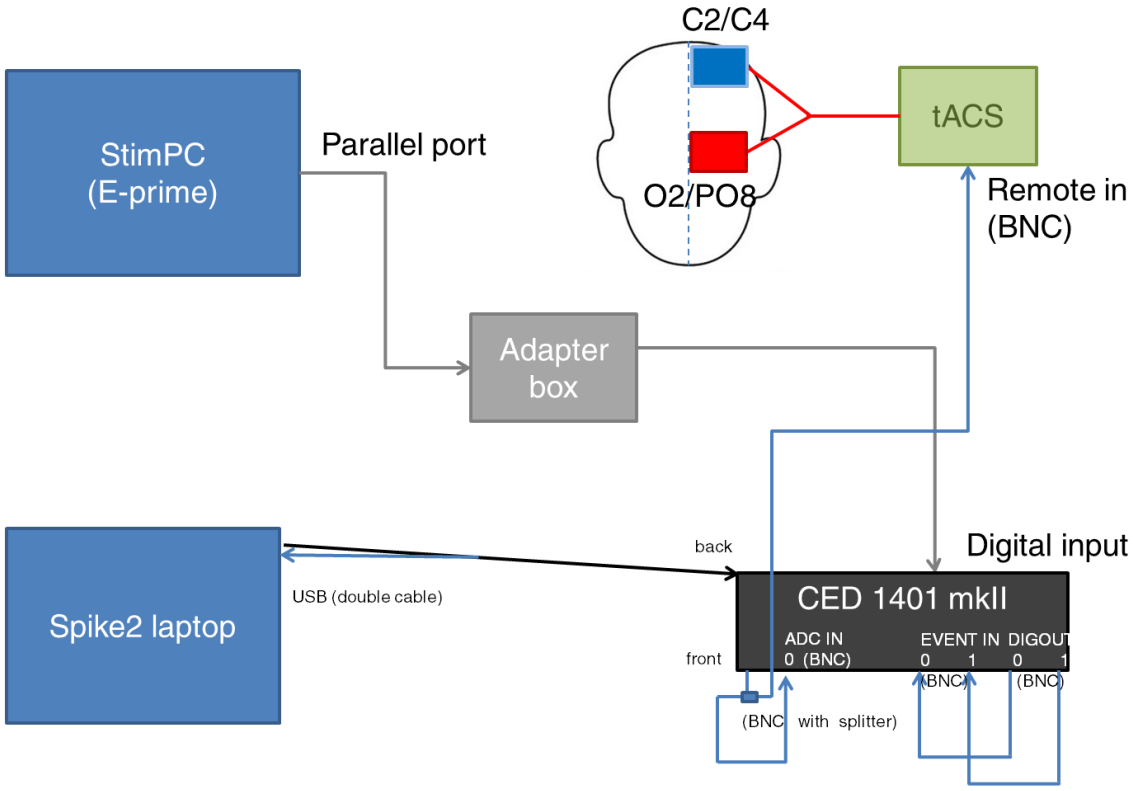

Figure 4.3: Experimental hardware setup.

participant, ranging between 0.9 and 2.0 mA (peak-to-peak amplitude/pp; *M* = 1.40, *SD* = .34, see Table 4.1). There were three tACS conditions (Figure 4.2, bottom): *Alpha* (stimulation at IAF), *Gamma* (stimulation at 40 Hz), and *no tACS* (no stimulation). The tACS-waveform was controlled through Spike2 software via a Power1401 mkII microcomputer (both Cambridge Electronic Design, Cambridge, UK), which was in turn controlled by E-prime stimulus presentation software (Psychology Software Tools, Sharpsburg, USA; Figure 4.3). On each active trial, the amplitude of tACS at the appropriate frequency was ramped up over 1 s to alleviate associated skin sensations and then remained constant until 300 ms after target onset or for maximally 2 s in case of catch trials when no target was shown. Stimulation was switched off for at least 1.4 s between trials. Experiment 1 showed that entrainment effects on brain oscillations, if present, do not outlast tACS offset even for as briefly as 200 ms, thus carryover entrainment effects are unlikely with this inter-tACS interval. Total stimulation time was approximately 15 min over the course of an hour. The waveforms including time stamps for the onset of visual targets were recorded at a sampling rate of 5 kHz and stored for offline analysis of phase dependence.

Statistical analysis

Statistically, the group results of accuracy and reaction time for unilateral targets were tested using a repeated measures ANOVA with factors Stimulation with three levels (No tACS, Alpha, Gamma) and visual field of target presentation (VF) with two levels (Left, Right). Analyses were conducted in IBM SPSS Statistics (version 21). When univariate test results involve more than one degree of freedom (i.e., factors with more than two levels), Greenhouse-Geisser-corrected p-values and the corresponding corrective epsilon (ε) values are reported to account for possible violations of sphericity. Effects sizes are indicated by Cohen's *d* for paired t tests and partial eta squared (η^2) for repeated measures analysis of variance (rmANOVA). Statistical analysis of phase dependency was conducted in MATLAB using the Rayleigh test as implemented in the circ_rtest function in the CircStat toolbox (Berens, 2009).

Results

Due to errors during data acquisition, for three participants the dot sizes for unilateral and bilateral conditions were not identical. Therefore these analyses are presented separately.

Titration

The median dot size chosen was 6 pixels for unilateral and 7.5 pixels for bilateral trials, with a range of 4 to 16. 14 out of 20 volunteers showed a slight to moderate right-sided advantage, with higher performance scores for dots presented in the right hemifield. Median performance on catch trials (in percent correct rejections) was 100%, however with a range of 9 to 27 (of 27) correct rejections because three participants (S1/15/20) gave many false alarms.

Unilateral targets: Detection accuracy

Detection performance for unilateral targets are shown in Figure 4.4 (raw group data), Figure 4.5 (group difference scores), and Figure 4.6 (individual differences). Overall, participants performed more poorly in detecting left-sided stimuli, or alternatively, show a bias towards responding "right", reflecting the moderate bias already observed in the titration task. Considering each hemifield

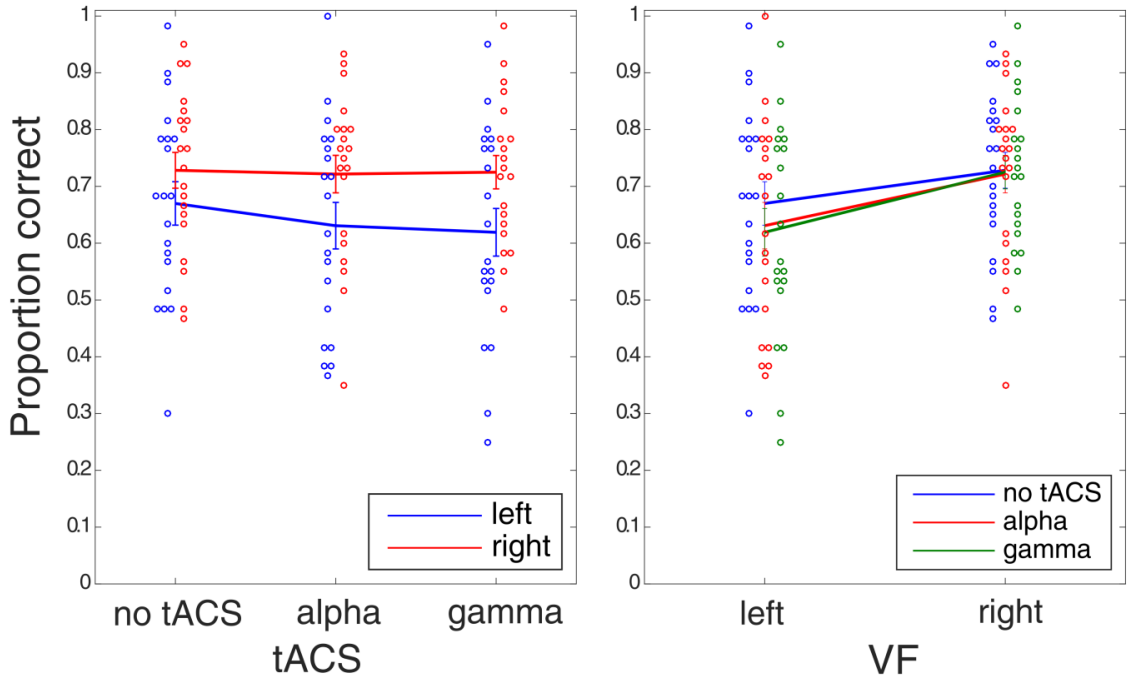

Figure 4.4: Detection performance for unilateral trials

Left: Performance (in proportion correct) grouped by tACS condition. *Right*: The same data grouped by visual field (VF) in which the target was presented. Circles represent individual participants, lines represent mean proportion correct, error bars represent standard error of the mean (N = 20).

separately, there was a tendency for lower accuracy with alpha stimulation compared to no tACS in 14 out of 20 participants for targets presented in the left visual field (VF), corresponding to a reduction of roughly 4% in detection performance for the whole group (see table Figure 3.2 for mean differences between conditions). In contrast, only 9 out of 20 participants showed deterioration in the right VF. Five participants showed improvement with α-tACS for left-sided targets compared to no tACS, compared to 9 participants for RVF targets. For γ-tACS, 14 out of 20 showed deterioration (with an approximate 5% reduction in average performance). Two participants showed improvement for targets in the LVF, compared to 11 improvements and 9 deteriorations for RVF targets, respectively. Thus, while tACS might have a (weak) negative impact on target detection in the expected direction for α-tACS, the similar pattern observed for γ-tACS suggest that this effect is not frequency-specific. Statistically, these results did not reach significance (Stimulation: $F(2,38) = 1.87$, $p = .18$, $\eta^2 = .09$, $\varepsilon = .81$; VF: $F(1,19) = 4.27$, $p = .053$, $\eta^2 = .18$; interaction: $F(2,38) = 2.05$, $p = .155$, $\eta^2 = .10$, $\varepsilon = .79$), although exploratory paired t-tests

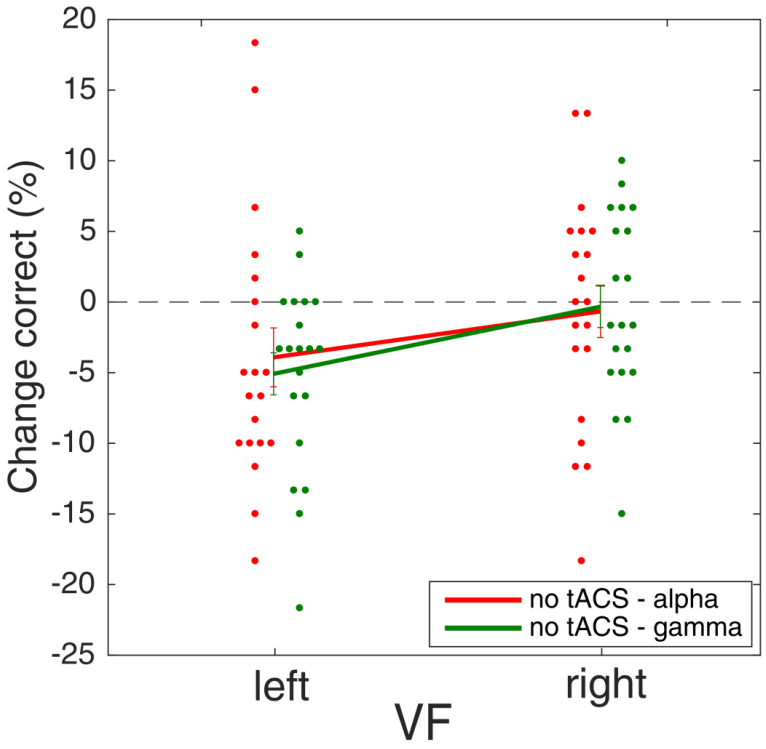

Figure 4.5: Detection differences in unilateral trials with and without tACS

VF = visual field. Circles represent individual participants, lines represent mean proportion correct, and error bars represent standard error of the mean (*N* = 20).

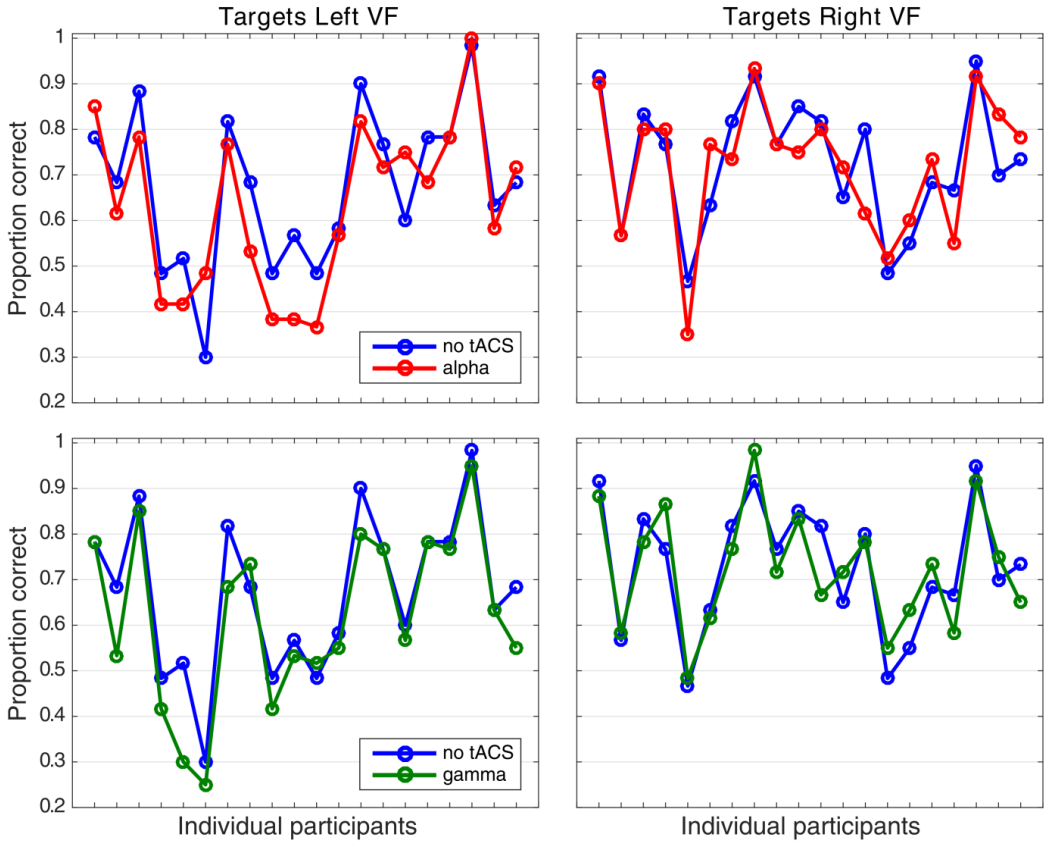

Figure 4.6: Individual detection results for unilateral trials

Top and bottom row show results for alpha- and gamma-tACS, respectively. Left and right column show results for targets in left and right visual field (VF), respectively. Connecting lines are added for ease of visualisation.

Table 4.2: Differences in dot detection accuracy in unilateral trials

Paired t-tests, df = 19, 2-tailed, uncorrected.

Comparison	*% mean difference (SD)*	*p-value*
Left VF		
No tACS vs Alpha	3.9 (9.3)	.075
No tACS vs Gamma	5.1 (6.7)	.003
Alpha vs Gamma	1.2 (11.6)	.657
Right VF		
No tACS vs Alpha	0.7 (8.3)	.722
No tACS vs Gamma	0.3 (6.6)	.823
Alpha vs Gamma	-0.3 (8.3)	.860

support a trend for lower performance in α- and γ-tACS trials compared to no tACS trials only for targets presented in the left VF (see Table 4.2). However, they also confirm that there is no difference between the different active protocols.

Unilateral targets: Reaction time

Median reaction time was calculated per participant and condition from all correct trials with a reaction time greater than 150 ms (assuming false alarms otherwise). The distribution of median reaction times was very similar across conditions (Figure 4.7), which is also reflected by the statistical test which shows no significant effects of either Stimulation ($F(2,38) = .96$, $p = .39$, $\eta^2 = .05$, $\varepsilon = .92$), VF ($F(1,19) = .60$, $p = .45$, $\eta^2 = .03$), or an interaction thereof ($F(2,38) = .37$, $p = .68$, $\eta^2 = .02$, $\varepsilon = .93$). Hence, tACS did not affect the speed of response.

Unilateral targets: Time course of detection performance

It is possible that tACS induces plastic changes that develop over time and that could affect excitability and therefore visual processing. The impact of such changes should increase with time into the experiment. Averaged over the whole experiment, these changes could mask event-related tACS effects. Figure 4.8 shows the average accuracy across participants over the course of the ten blocks. Interestingly, performance on average seems to decline over time only for targets presented in the left hemifield while the hit rate remains relatively

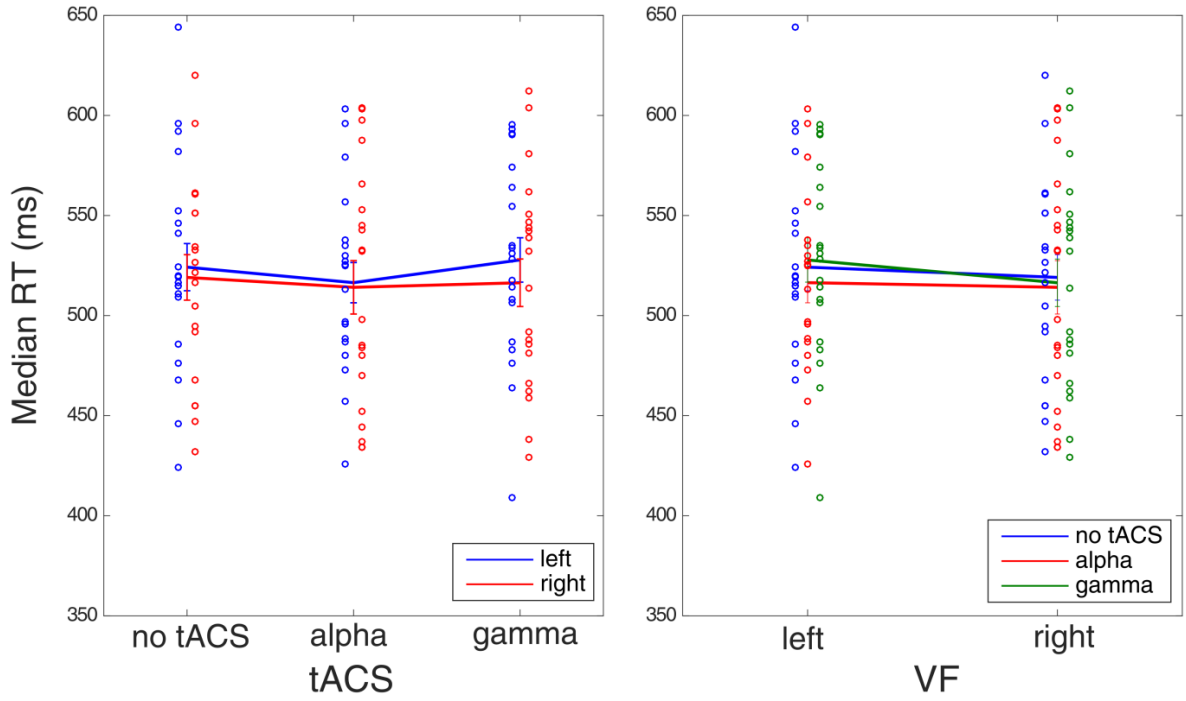

Figure 4.7: Median reaction time for unilateral trials

Left: Performance grouped by tACS condition. *Right*: The same data grouped by visual field (VF) in which the target was presented. Circles represent individual participants, lines represent mean median RT, and error bars represent standard error of the mean (*N* = 20).

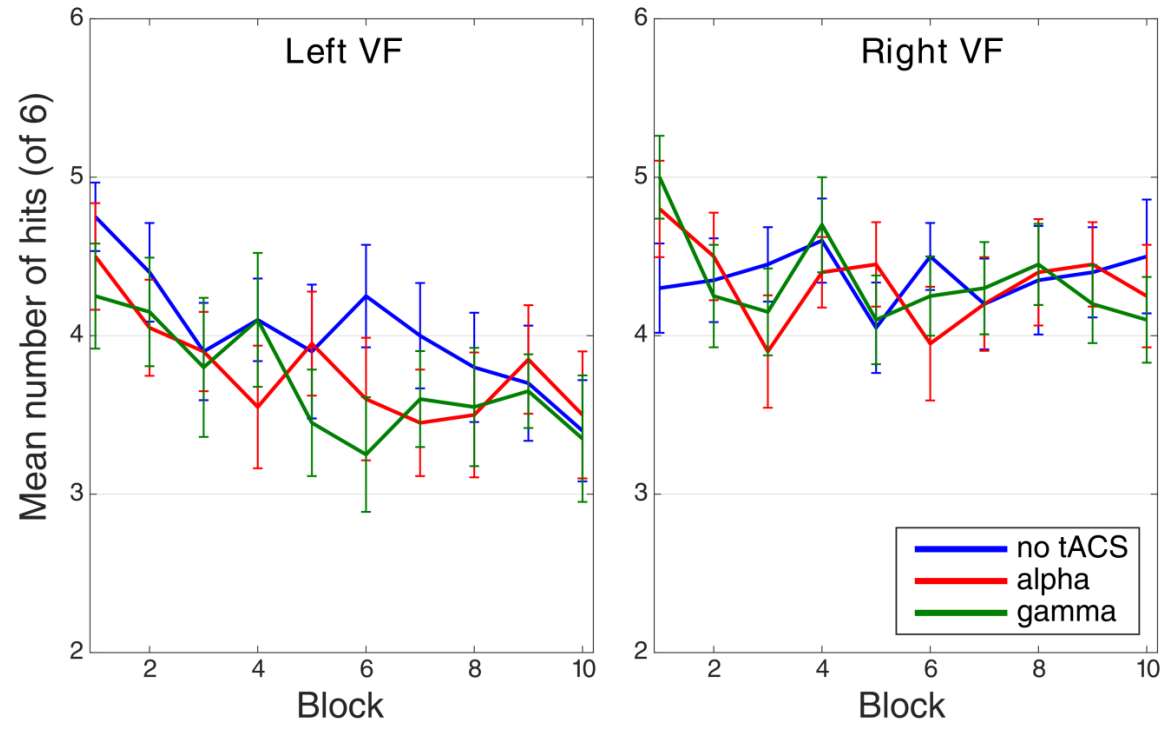

Figure 4.8: Time course of hit rate in unilateral trials

The mean number of hits for unilateral targets seems to decrease more quickly over the ten blocks for tACS trials compared to no tACS trials and only for targets presented in the left visual field (VF). Error bars represent standard error of the mean (*N* = 20).

constant for right-sided targets. In addition, the plot suggests that detection accuracy deteriorates more quickly for trials with tACS. Statistically the differences in time course were tested by taking the sum of correct detections across three successive blocks (excluding the first block), corresponding to eighteen targets per condition. A repeated measures ANOVA with factors Block (Early, Middle, Late), Stimulation (No tACS, Alpha, Gamma), and VF (Left, Right) yielded a near-significant main effect of Block ($F(2,38) = 3.21$, $p = .055$, $\eta^2 = .15$, $\varepsilon = .94$), and a significant effect of VF ($F(2,24) = 4.42$, $p = .049$, $\eta^2 = .19$), yet neither the effect of Stimulation, nor of any interaction, were statistically discernible (Stimulation: $F(2,38) = 2.289$, $p = .12$, $\eta^2 = .11$, $\varepsilon = .88$; Block x VF: $F(2,38) = 2.45$, $p = .10$, $\eta^2 = .11$, $\varepsilon = .95$; all other $F < 1$). Post-hoc t-tests support a slight drop in performance from early blocks to middle blocks corresponding to a mean of 0.6 missed targets per (concatenated) block ($p = .054$ and .051 from the early to the middle and late blocks, respectively). On average, 1.6 targets more were missed on the left compared to the right VF. In the absence of interactions of Block and/or VF with Stimulation, and a corresponding consistent pattern across participants (Figure 4.9), the notion of accelerated decline in performance in active tACS trials reflecting an interaction of online and plastic effects is therefore not supported by the statistical test. However, the possibility of an overall accumulating plastic or excitability change that affects performance on all trials equally cannot be fully ruled out by the data.

Bilateral targets: Detection accuracy and error analysis

If detection performance was decreased contralateral to stimulation, participants should be more likely to report bilateral targets as unilateral targets ipsilateral to stimulation. More specifically, instead of detecting bilateral dots there should be a response shift towards right VF responses and away from left VF responses. The distribution of bilateral errors is shown in Figure 4.10. One participant (S10) consistently gave right responses to bilateral targets or missed them entirely (almost certainly because the size for bilateral targets had accidentally not been adjusted) so was excluded from this analysis. There was no difference in accuracy on bilateral trials between the different tACS conditions ($M_{\text{no tACS}} = .67$, $SD = .18$; $M_{\text{alpha}} = .68$, $SD = .18$; $M_{\text{gamma}} = .66$, $SD = .20$; repeated measures ANOVA with factor Stimulation: $F(2,36) = .20$, $p = .80$,

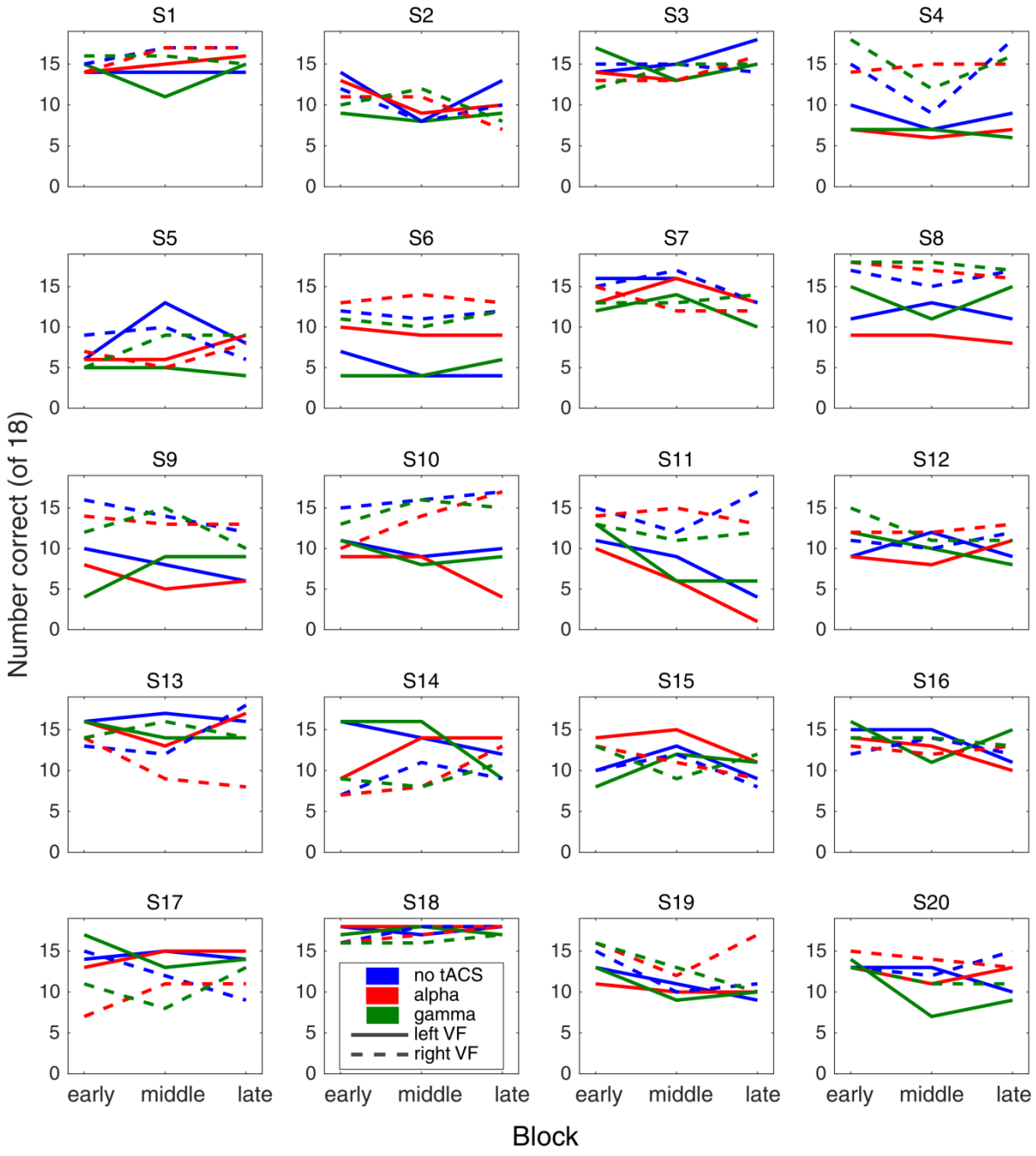

Figure 4.9: Individual hit rate time courses

Number of hits is averaged over early, middle and late trials (blocks 2 - 4, 5 - 7, and 8 - 10, respectively) for targets presented unilaterally in the left (solid lines) and right (broken lines) visual fields (VF).

η^2 = .01, ε = .92; see also right lower quadrant in Figure 4.10). The mean number of specific errors (including left responses, misses, and right responses) did also not differ between tACS conditions (separate rmANOVAs; left responses: $F(2,36) = 2.06$, $p = .16$, $\eta 2 = .10$, $\varepsilon = .75$; all other $F < 1$).

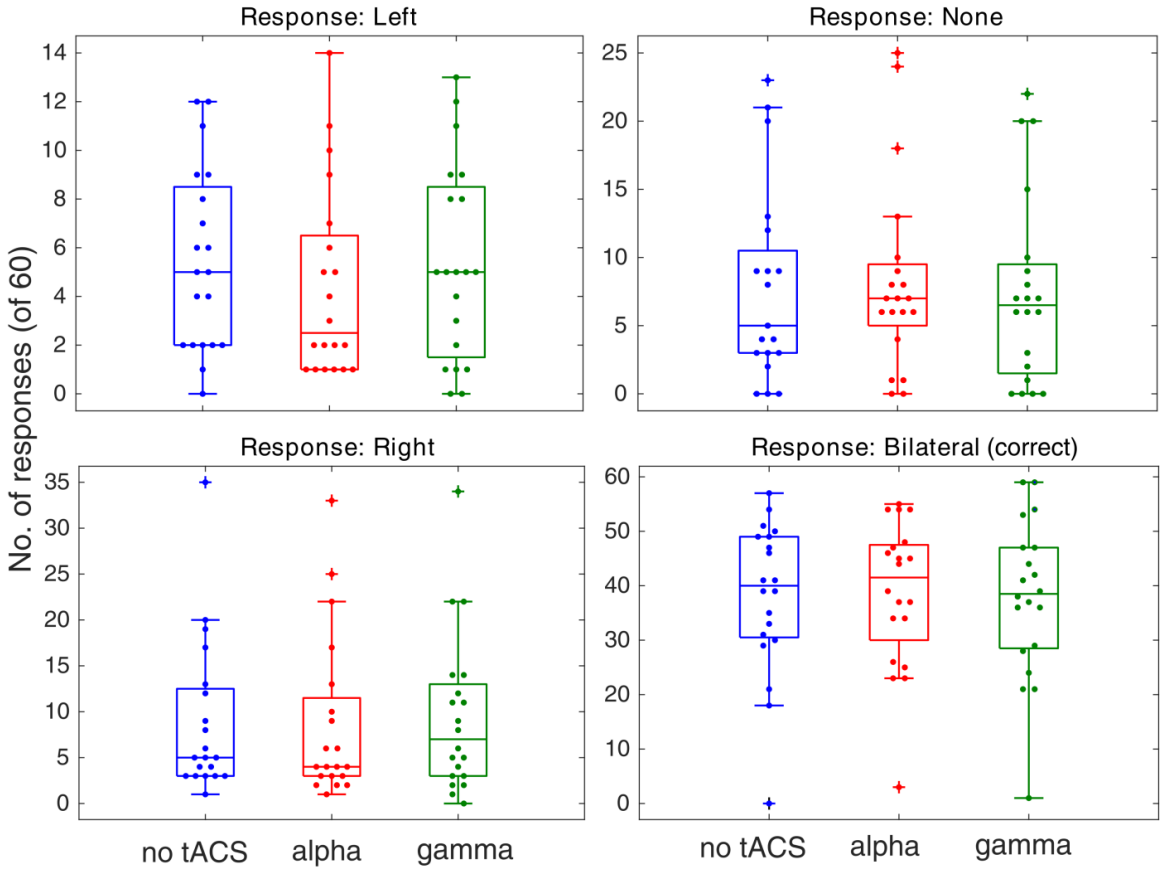

Figure 4.10: Responses to bilateral targets across tACS conditions

Dots represent individual participants. The left column shows erroneous unilateral responses; the right column shows misses (top) and correct bilateral responses (bottom). Note that the bottom data point in each tACS-condition in the lower right quadrant for correct bilateral responses belong to a participant who was not included in the statistical analysis because of near-zero correct identification of bilateral stimuli.

To account for the interdependence of changes in bilateral versus left and right responses, possible response shifts were additionally analysed following Hilgetag et al (2001) by calculating relative composite vectors from both correct and incorrect responses to uni- and bilateral targets (Figure 4.11). These response vectors give an indication of the deviation from perfect detection performance towards either the left or right hemifield. The respective proportions of correct responses (e.g., "bilateral" for bilateral targets) and incorrect responses (according to this example, the proportion of "right" responses and the proportion of "left" responses) were calculated for each of the six (tACS by VF) conditions separately. The proportion of "left" responses was then subtracted from the "right" responses. Thus, for each level of VF we obtained a bilateral component (*B*) and a left-right component (*R*-*L*). A value of *B* between 0 and 1 for bilateral responses is equivalent to 0 - 100% correct detection of bilateral targets, depending on the number of misses and incorrect

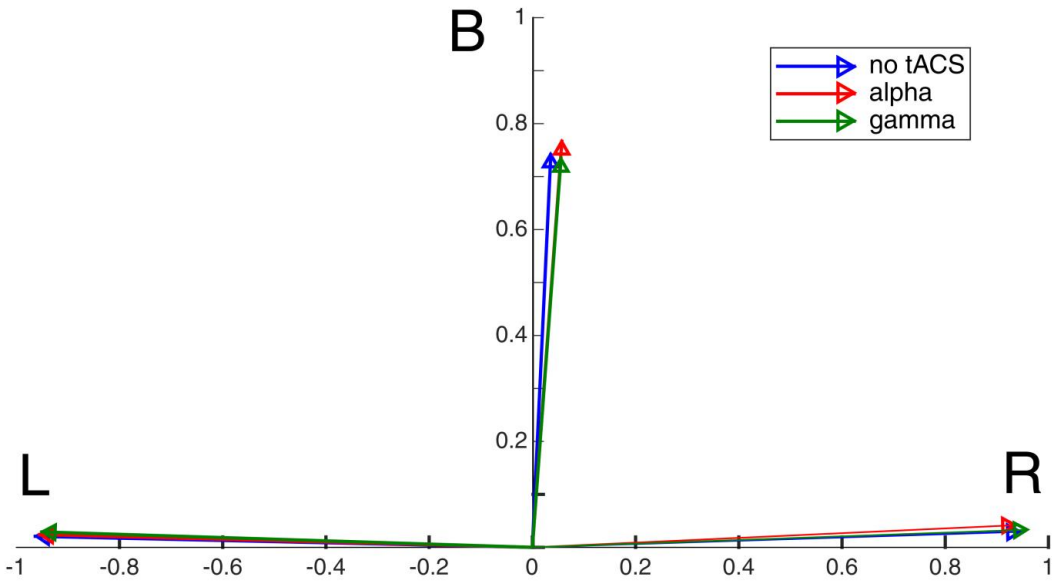

Figure 4.11: Response vectors for bilateral targets

The mean response vectors (N = 19) quantify the response bias towards a particular hemifield in the different tACS conditions. Left-pointing arrows are obtained from left unilateral target trials, right-pointing arrows from right unilateral target trials, and upward-pointing arrows from bilateral trials. The magnitude along the abscissa (B-axis) reflects the relative proportion of (correct and incorrect) unilateral versus bilateral responses. The magnitude along the ordinate (R-L axis) reflects the relative proportion of (correct and incorrect) left versus right responses. A value of |1| on each axis (L, R, B) reflects 100% detection accuracy. Note that in this plot, the response vector magnitude is not adjusted for misses, i.e., only takes into account trials on which a response was given.

responses. R-L can assume values between -1 (that is, 100% correct detection of left VF targets) and 1 (100% correct detection of right VF targets) for unilateral responses. These values determine the length (magnitude) and direction of the response vector.

Analysis of the unilateral component (R-L) for bilateral trials (i.e., the left-right deviation from the abscissa) using one-sample t-tests do not support a significant deviation from zero in any tACS condition, reflecting the lack of a attentional bias towards targets in the right hemifield at the exclusion of targets in the left hemifield (all $p > .11$, 2-tailed, uncorrected and independent of whether response vectors are adjusted by including misses in addition to incorrect responses in the calculations). There was also no difference in the R-L component between tACS conditions ($F < 1$ for adjusted and unadjusted response vectors). Finally, there was no difference between the bilateral components (i.e., the length of the bilateral vector along the abscissa) of each tACS condition for either adjusted or unadjusted response vectors (unadjusted: $F(2,38) = 1.56$, $p = .26$, $\eta^2 = .076$, $\varepsilon = .89$; adjusted: $F < 1$). In sum, we found no

evidence for a change in spatial bias in trials with bilateral targets toward the right hemifield as would be consistent with improved right hemifield detection or impaired left hemifield detection.

Phase dependency of detection accuracy

If tACS phase has a modulatory effect on detection performance that mimics the dependency on endogenous alpha phase, cyclic changes in detection accuracy should be observable for left (contralateral) VF targets but not right VF (ipsilateral) targets. Therefore, hits and misses should be associated with different (if not opposite) phase angles in different sections of the sine wave, i.e., show an out of phase distribution. This phase segregation should be more obvious, if not exclusive, to targets in the left VF. To assess this hypothesis, tACS phase was extracted from the recorded continuous tACS waveform using the following pipeline. First, events corresponding to target presentation within the different trial types (left/right/bilateral targets during alpha- or gamma stimulation) were identified based on the log files generated by E-prime. 'No tACS' and catch trials were not considered due to the absence of a waveform in the former, and the absence of a target in the latter. Based on the reconstructed time stamps, epochs were extracted from 400 ms before to 300 ms after target onset, corresponding to a portion of the signal with a stable sine wave. The instantaneous phase angles (in radians) at the time of target onset were calculated from the complex Hilbert transform of each epoch.

For each participant and condition, trials were sorted by hits and misses and the distribution plotted as a function of phase angle (Figure 4.12). From the individual data we can make three observations: First, none of the participants shows the expected pattern of a clear phase separation between hits (green data points) and misses (red data points): Neither hits nor misses cluster at any particular phase angle or region, and in any case not in separate regions, as indicated by a lack of an alternating red-green pattern within each condition. Second, the distribution of hits does not obviously differ between left and right VF targets (see αL versus αR and γL versus γR). Third, other than a greater phase density for γ-trials (resulting from the greater similarity between the refresh rate and tACS frequency, see next paragraph), the distribution of correct and incorrect trials does not strongly differ between α- and γ-trials.

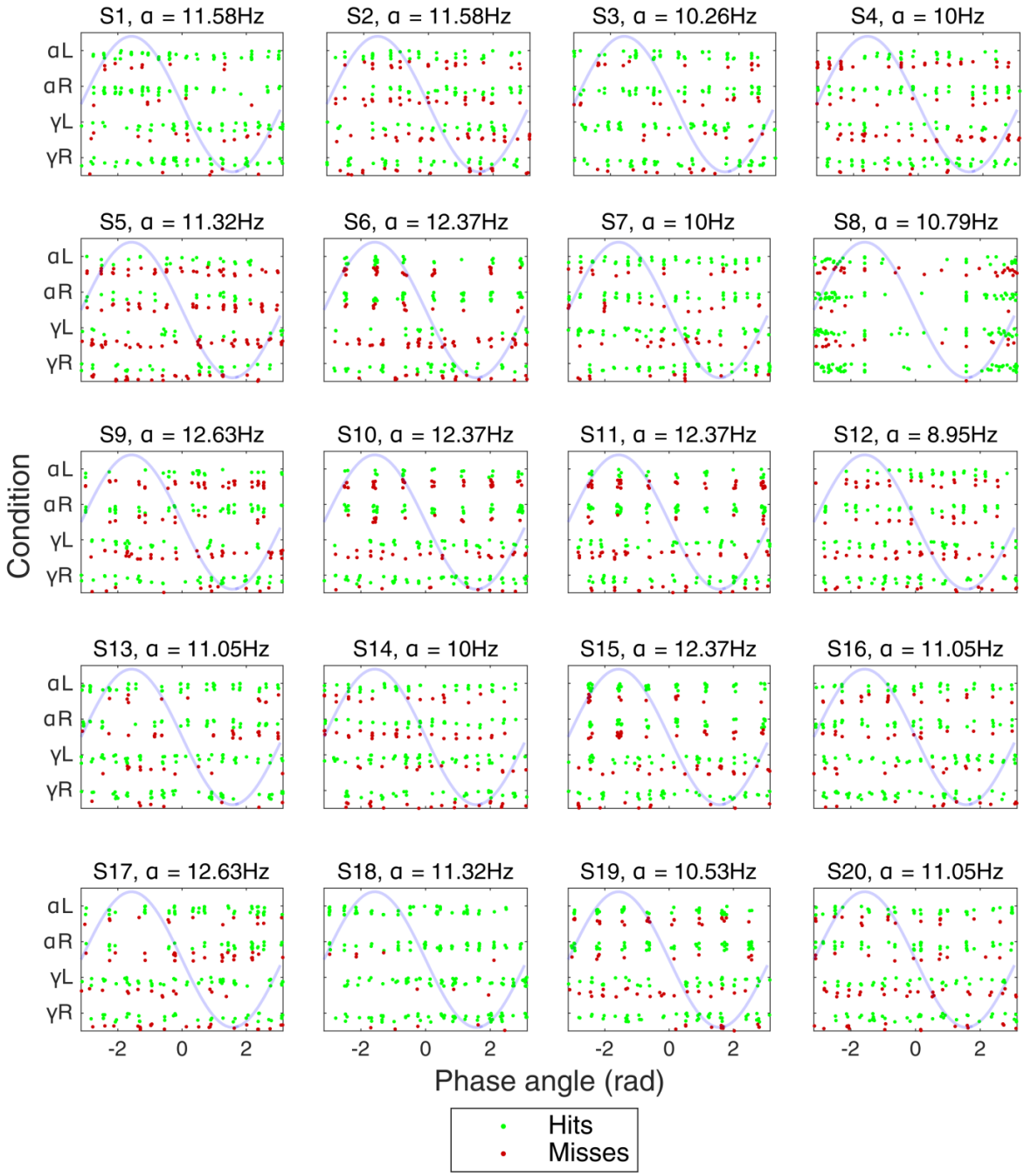

Figure 4.12: Dependence of detection accuracy on tACS phase

Plots show the distribution of correct and incorrect unilateral target trials as a function of tACS phase angle (in radians) for individual participants. Green and red horizontal bands of dots represent hits and misses/incorrect responses in each of the four conditions (tACS frequency: alpha α or gamma γ; visual field: left L or right R). If detection accuracy, particularly in the left VF, depended on α-tACS phase, we should be able to observe a separation between the distributions of hits (green dots) and misses (red dots) along the waveform, particularly in the top row of each individual subplot. Such a separation is not obvious in any of the distributions of left and right VF targets in α- or γ-trials, thus none of the participants showed the expected phase dependency. Scatter along the vertical dimension is added for better visibility. Note that the requested alpha stimulation frequencies were dependent on the stimulator resolution (roughly 0.26 Hz) and were estimated empirically for phase analysis. γ-frequency (both requested and delivered) was 40 Hz.

There are a number of problems that are evident from Figure 4.12 which, however, do not invalidate the above observations. Firstly, the number of incorrect trials is generally lower than the number of correct trials, which makes the appearance of a non-uniform distribution more likely. Secondly, the number of trials per condition (N = 60) was too low to adequately ensure an even distribution of target presentation per phase angle across the whole cycle with random jitter. In other words, some regions in phase space contain inherently fewer trials than others. Thirdly, several participants show clear bin-like patterns, rather than a random distribution including all possible phase angles. As examples, consider the data from participants S6, S10, or S19 in Figure 4.12. The top rows corresponding to alpha trial phases fall into discrete bands, compared to the more uniform distribution of for instance S1 and S12. These patterns appear to be a function of tACS-frequency. On closer inspection, they can be explained at least in part by the fact that both the initiation of the tACS waveform and the target presentation (and its associated time stamp) were coupled to the screen refresh rate. Figure 4.13 shows the actually measured target phases and the predicted phases when time-locking to the monitor refresh rate (85 Hz) is taken into account, that is when targets are presented across an interval of 200 ms but "binned" into refresh rate intervals. This plot shows that the pattern of recorded phases and predicted phases vary as a function of stimulation frequency but are remarkably similar within most data sets (ignoring possible phase offset; compare in particular the red triangles (predicted) and blue dots (recorded) for alpha targets, corresponding to the red sine wave labelled "IAF"). An exception is participant S8, where the target phases are clustered within one half-cycle; it is unclear why only few targets were presented in the other half cycle.

Given these problems, a formal statistical analysis is unlikely to provide meaningful guidance on whether there exists a preferred (or abhorred) phase for target detection. Regardless, the phase distribution in the Alpha condition was tested statistically using the Rayleigh test which tests whether circular data follow a uniform distribution. This test assumes that the phase distribution is at most unimodal (i.e., clusters around one direction if non-uniform) and that phases are sampled from a von Mises distribution, which can be thought of as the equivalent of the normal distribution on a circle. To avoid biasing the results by

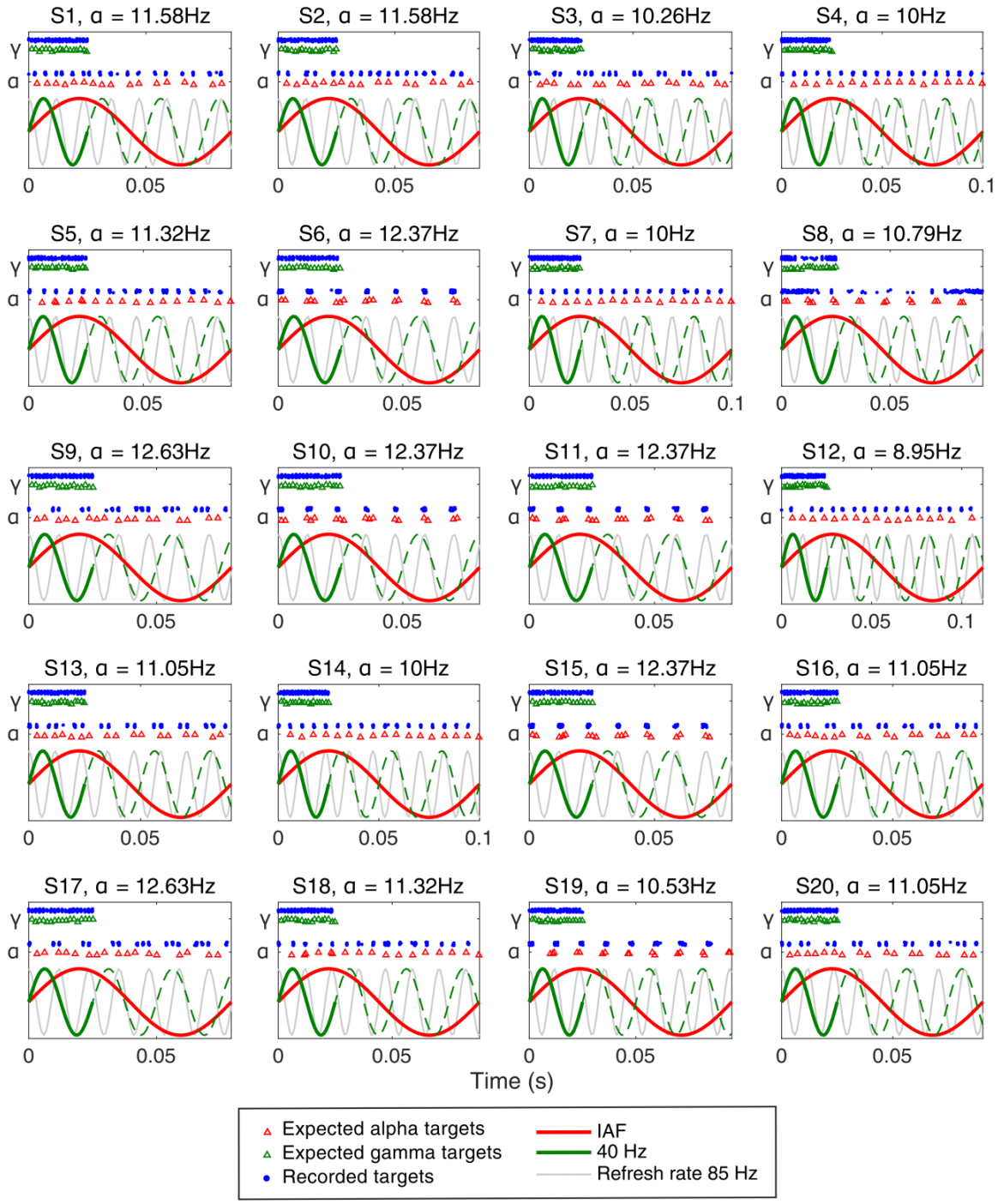

Figure 4.13: Bin-like appearance of phase distribution

The uneven phase distribution is driven by time-locking to screen refresh rate.

unequal sample sizes, a random sample of trials was selected from each of the four conditions (that is, from hits/misses of left/right VF targets, respectively). The size of the sample depended on the respective minimum number of correct or incorrect trials for left or right targets per participant. Participants with less than 13 trials (which has been found to be the minimum for reliable results on this test; Durand & Greenwood, 1958) in at least one of the four conditions

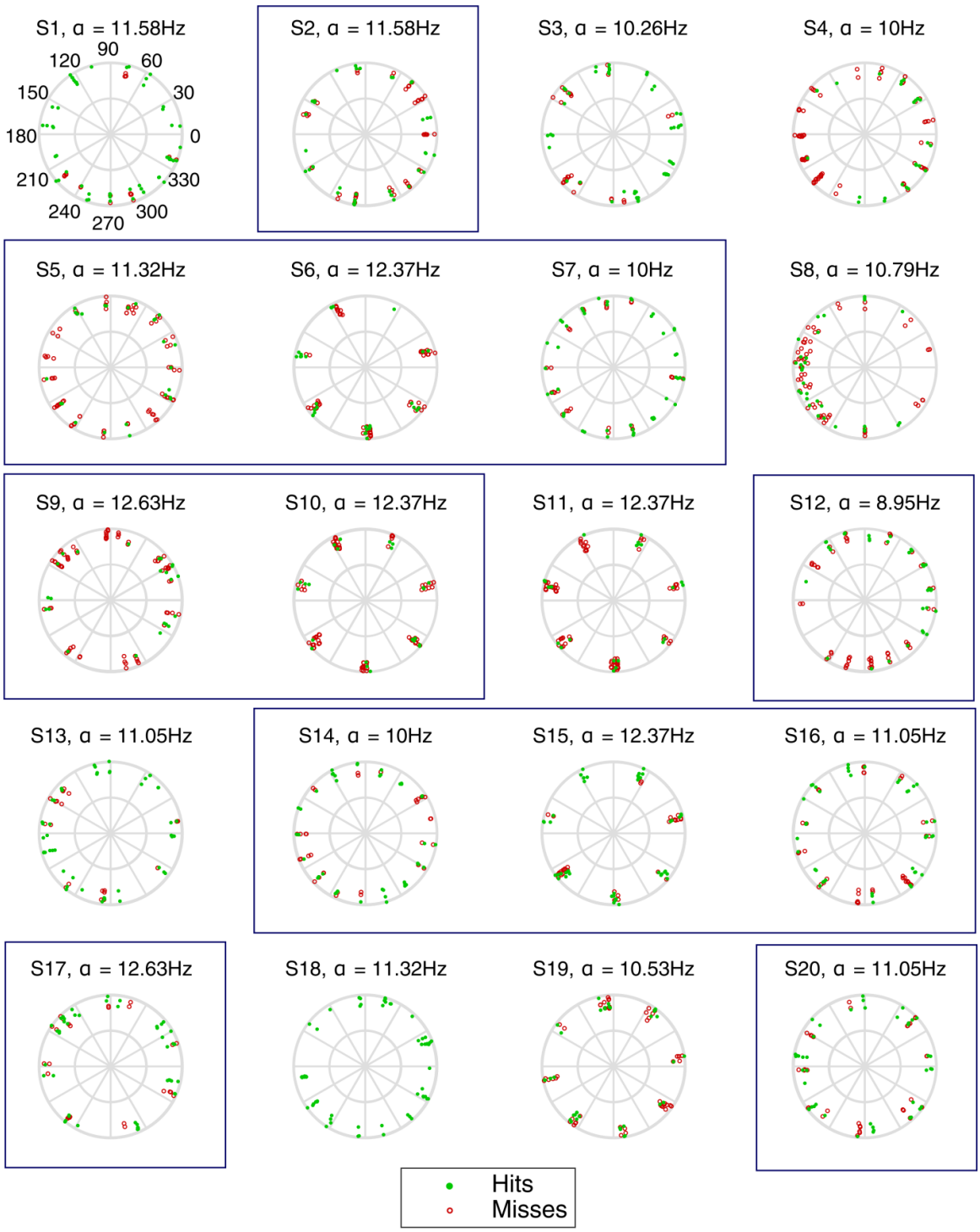

Figure 4.14: Phase distribution of hits and misses

There is no preferred phase for hits and misses in the left visual field during alpha tACS. Note that these are polar representations of the data in the top row of each participant in Figure 4.12. Blue squares indicate participants who were included in the statistical analysis.

were excluded from this analysis (*N* = 8), leaving twelve data sets (S2, S5 - 7, S9 - 10, S12, S14-17, S20). Separates tests were run for each of the four conditions for each participant individually. As can be expected based on Figure 4.14, which shows the phase distribution for left VF targets during α-tACS,

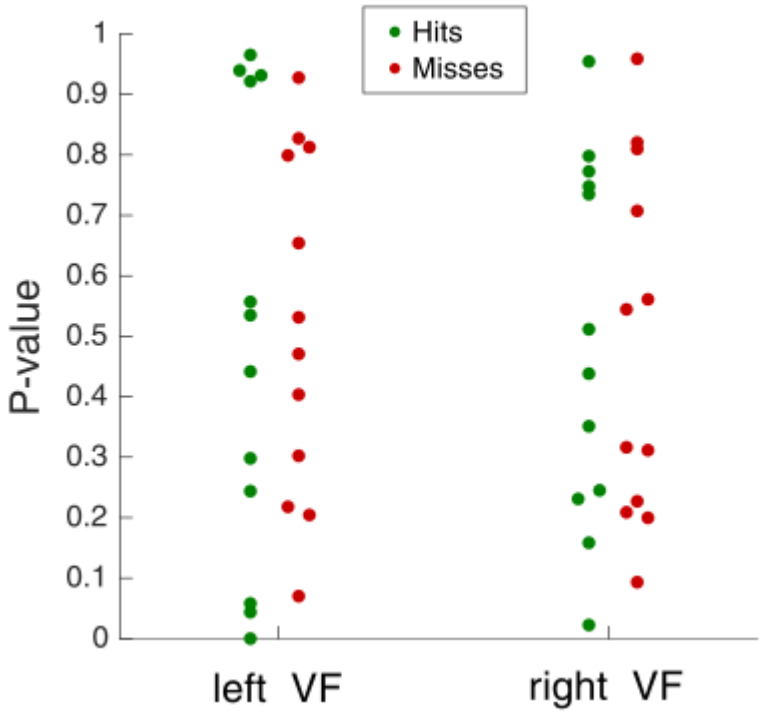

Figure 4.15: Rayleigh test results for non-uniformity of circular data

The distribution of uncorrected p-values of individual tests per participant does not suggest that the phases of correct and incorrect trials are non-uniformly distributed.

the distribution of test outcomes does not support non-uniformity of the data (Figure 4.15). Therefore, taken together, the relationship between (intrinsic) α-phase and visual detection performance was not observable for tACS at α-frequency.

Rating of peripheral sensations

The mean ratings for the intensity of skin sensations, discomfort, and visual anomalies provided between the ten experimental blocks are shown in Figure 4.16. Moderate to high ratings (> 3) were more commonly reported in the first half of the experiment, particularly for skin sensations and discomfort, suggesting that at least those participants that perceived uncomfortable scalp sensations initially became less sensitive over time. This notion is supported by voluntary statements by the participants during and after the experiment and statistically by significant Friedman tests on the average rating of the first, middle, and final three blocks, respectively (skin sensation: $X^2(2) = 19.4$, $p < .001$; discomfort: $X^2(2) = 8.52$, $p = .014$). Follow up Wilcoxon Signed Rank tests confirm a decrease in perceived strength over time (skin sensation: early versus middle block $Z = 3.71$, $p < .001$; early versus late block $Z = 3.44$, $p < .001$;

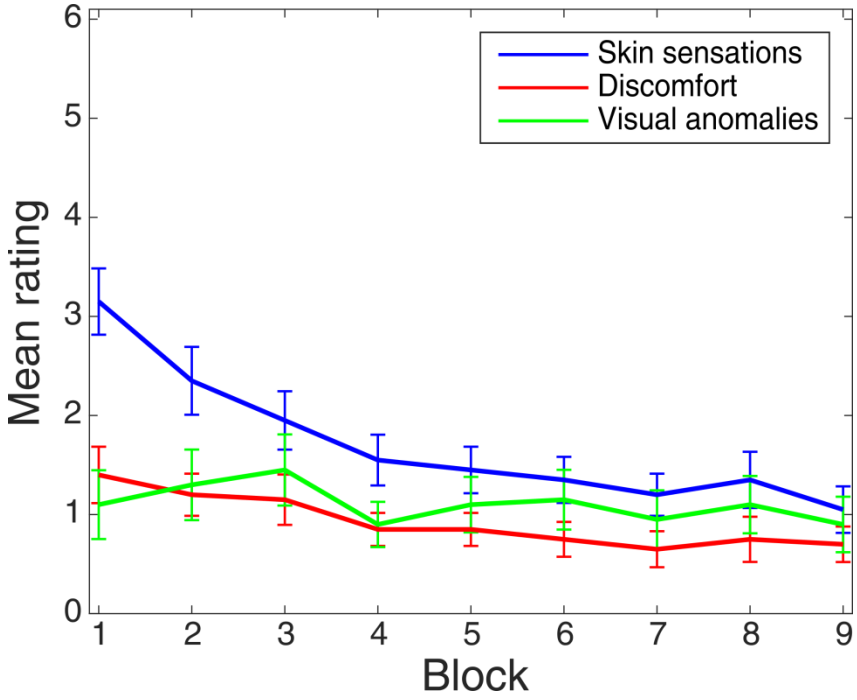

Figure 4.16: Intensity of skin sensations, discomfort, and visual anomalies

Mean ratings across the nine experimental blocks. Error bars represent standard error of the mean (N = 20).

middle versus late block p = .076). Discomfort: early versus middle block Z = 2.71, p = .007; early versus late block Z = 2.09, p = .037; middle versus late block p = .48). For visual sensations, the ratings were more constant, with no systematic differences between blocks ($X^2(2)$ = 2.44, p = .30). Despite the intensity adjustment before the experiment, some participants still experienced "wobbling" and flickering phosphenes, with a couple of people describing a feeling of increased pressure to the eyes. However, the ratings reflect at least partly the strain of fixating at a bright screen for an extensive time period. The heightened sensitivity despite generally lower stimulation intensities is in contrast to experiments 1 and 2 and probably reflects the different montage.

Discussion

This experiment aimed at addressing the limitations in Brignani and colleagues' study (Brignani et al., 2013) and thereby increasing the likelihood to detect changes in visual spatial perception that could theoretically be expected if α-tACS can entrain, or at least mimic, intrinsic α-activity. As hypothesised, average detection accuracy in a peripheral dot detection task was slightly worse for targets presented contralaterally to tACS (that is, in the left visual field/VF) during α-stimulation compared to tACS-free trials. However, a similar decline in

performance was also observed for trials with γ/40 Hz stimulation. Overall, this effect was too weak to be statistically significant. tACS had no effect on the detection of targets presented either ipsilaterally to tACS (that is, in the right VF) or bilaterally. tACS also did not affect median reaction times, which suggests that stimulation did not interfere with response preparation. Finally, the phase of α-tACS was not predictive of successful stimulus detection, contrary to the findings of phase dependence of visual performance with respect to endogenous α-oscillations (Mathewson et al., 2011; VanRullen et al., 2011). Taken together, the present data do not support the idea that α-tACS can be used to manipulate visuospatial attention and stimulus processing by entrainment of lateralised alpha activity. Note that the current design explicitly assumes a causal influence of lateralised alpha in shaping a visuospatial bias (Romei et al., 2010), that is, we assume that tACS acts by emulating the brain's own mechanisms. Accepting that the weak effect on detection in the contralateral VF is real, the lack of frequency- and phase-specificity casts doubt on the notion that tACS induces its online effects exclusively by entrainment of intrinsic oscillations.

These results are not incompatible with those of Brignani and co-workers (2013, summarised in the introduction above). The observed impact in their study was only weakly frequency-specific and did not involve immediate visual processing but rather reflected a disruption of perceptual learning in that participants failed to improve on their detection task after 6 or 10 Hz tACS. In the absence of online neural data, it is not possible to deduce that such failure to improve is a consequence of neural entrainment.

Like this previous study, this experiment has a number of limitations that do not allow strong conclusions. The left VF tACS-effect was too weak to be statistically significant and needs to be treated as preliminary finding. However, with only a small sample size and in the light of converging results for α-and γ-tACS we might be allowed to attribute this lack of significance to lack of statistical power. For the sake of argument I am therefore going to accept that the decline in detection performance for targets in the left VF is a true consequence of tACS.

Could similar effects for Alpha and Gamma-tACS reflect entrainment of the same neural rhythms?

Opposite to our predictions, more participants showed decreased performance in trials with 40 Hz stimulation than with α-stimulation. Statistically, Alpha and Gamma performances were not discernible. This suggests that 40 Hz stimulation is at least as effective as α-tACS in producing a deficit that is consistent with a spatial bias away from the hemifield contralateral to tACS, despite the known inverse relationship and putative opposing roles of alpha and gamma oscillations in visual processing. It has been shown, at least in theory, that tACS at multiples of the intrinsic frequency F_N can also entrain $F_{N,}$ as well as its harmonics (Ali et al., 2013). Although few participants showed a precise IAF of 10 Hz (of which 40 Hz is an exact multiple), IAF is an intrinsically volatile concept which is often difficult to measure precisely and reliably, and is better conceived as a dynamic range of frequencies. Assuming a sufficiently high intensity of cortical current, it is therefore possible that 40 Hz stimulation was effective in entraining subharmonic α-network activity. Interestingly, in the context of rhythmic visual stimulation it was shown that visual flicker at higher frequencies is often accompanied by a steady state visual evoked response (SSVEP) in the α-band, especially if alpha overlaps with the stimulation subharmonic (Herrmann, 2001). Vice versa, stimulation at α-frequency was often accompanied by harmonic responses. Notably, at the group level there was enhanced SSVEP responsiveness in multiple frequency bands with a flicker frequency of 39 Hz. If the neural response to rhythmic electrical currents behaves in a similar fashion as the response to rhythmic visual input, the equivalence of alpha and gamma tACS could be explained by a similar spectral fingerprint in terms of the harmonics and subharmonics engaged by stimulation.

An alternative explanation is that periodically adding energy to the network at any frequency could have led to resonance of the underlying network (alpha) oscillation, albeit without the strong phase relationship expected from entrainment. Given the lack of phase dependency, the latter mechanism is more consistent with the data. A frequency-independent account does not explain, however, why Brignani's tACS effects were limited to lower frequencies (6 and 10 Hz) and can only partly be explained by a strong alpha responsiveness to any form of stimulation. A similar idea of a generic α-response to prolonged

stimulation has been expressed before in the context of rTMS (Veniero et al., 2011).

Another possible (not mutually exclusive) explanation could be that tACS modulates cross-frequency interactions that are intrinsic to the network structure. At this point, only one study has explicitly investigated cross-frequency tACS-effects (Helfrich, Herrmann, Engel, & Schneider, 2015). In their re-analysis of existing data, they observed increased phase-amplitude coupling between alpha and gamma activity with 10 Hz stimulation but increased amplitude envelope coupling with 40 Hz stimulation, the latter being correlated with α-power suppression during γ-tACS (i.e., showing the anticipated antagonistic relationship between alpha and gamma; see also Helfrich, Knepper, et al., 2014). One could speculate that such cross-frequency dependencies produced the observed similar deficits by preventing normal functioning of the whole network, albeit through somewhat different mechanisms. The 25 Hz condition in Brignani's experiment may have been ineffective because of a lack of relevant cross-frequency interactions. The bottom line is that an important step to understand how tACS affects overt behaviour is to understand how it interacts not only with oscillations at the stimulation frequency but also across frequencies, and to rule out other broadband mechanisms (for example such as the excitability changes attributed to tDCS).

Does frequency matter?

The lack of frequency-specificity in the current design appears to be inconsistent with the tACS literature. Other behavioural studies using tACS as causal modulator of visual perception have shown results consistent with entrainment of visual cortical networks. For example, Cabral-Calderin and colleagues (Cabral-Calderin et al., 2015) found that tACS at 60 Hz, but not 10 or 80 Hz, affected the number of perceptual reversals during bistable perception. They concluded that neural 60 Hz oscillations play a functional role in resolving perceptual ambiguity. Cecere and co-workers used tACS to speed up or slow down volunteers' endogenous α-frequency to shrink or enlarge the temporal asynchrony for audiovisual stimulus presentation that is required to elicit an illusory percept (Cecere et al., 2015). Laczó and co-workers (Laczó et al., 2012) concluded that 60 Hz tACS over V1 reduced contrast detection thresholds

compared to sham, while tACS at 40 or 80 Hz was ineffective. As reviewed in Chapter 1, many other studies outside the visual domain come to similar conclusions of frequency-specific tACS effects that explicitly or implicitly assume entrainment or resonance to the alternating current. On the other hand, experiments in which TMS rather than tACS was used to target parietal cortex with the specific aim to modulate visuospatial attention have produced hemifield-specific deficits using a variety of frequencies, although α-rTMS might be most effective (Romei et al., 2010). For example, deterioration of detection accuracy in the contralateral field was observed following ten minutes of 1 Hz rTMS (Hilgetag et al., 2001), demonstrating a clear aftereffect. Capotosto and colleagues (Capotosto et al., 2012) found a bilateral negative impact on reaction times and accuracy after short 20 Hz pulse trains to right IPS administered during the anticipatory interval after a spatial cue. This bilateral deficit was, however, accompanied by a paradoxical right-dominant event-related α-synchronization. Although both their behavioural paradigm (a cued attention letter discrimination task) and stimulation method differ from the current experiment, these results are potentially interesting: tACS at both alpha and gamma frequencies may have interfered in a similar, non-specific fashion by preventing event-related desynchronization particularly in the right hemisphere, thus producing a virtual left hemifield attentional deficit.

Modulation of spatial attention after stimulation of right PPC has also been reported after tDCS (i.e., 0 Hz). However, results have been mixed. Anodal tDCS may both improve (Roy, Sparing, Fink, & Hesse, 2015; Sparing et al., 2009) or weaken (Filmer, Dux, & Mattingley, 2015) processing in the contralateral VF, or have no effect (Loftus & Nicholls, 2012). Cathodal tDCS to right PPC tends to produce effects in line with an attentional shift away from the contralateral VF (Sparing et al., 2009; Benwell et al., 2015; Giglia et al., 2011), especially when combined with anodal stimulation to the left PPC (Giglia et al., 2011). However, Benwell and colleagues found this result only in subsets of their sample when split based on a combination of baseline performance and stimulation intensity. These mixed results need refinement and will at least partly be due to different tasks and stimulation parameters but they indicate that periodic stimulation is not necessary to produce behavioural changes.

The variable effects of the different NIBS protocols targeting right PPC on visuospatial task performance point to the possibility that the effects of tACS may have to be regarded not as frequency-specific but task-specific, encompassing both the activity of interacting inhibitory and excitatory networks, which can be distributed asymmetrically across hemispheres. In other words, while entrainment or resonance may be instrumental in producing some activity modulation for some neural subpopulations, the actual changes at the neural level that are causal to behavioural changes are likely more complex than a simple entrainment hypothesis predicts.

Lack of phase dependency

The lack of phase dependency is in contrast to studies that involved auditory, rather than visual detection tasks (Neuling, Rach, et al., 2012; Riecke, Formisano, et al., 2015; Riecke, Sack, et al., 2015). Despite the limitations introduced by non-uniform phase sampling in the current implementation, it is implausible that the absence of phase modulation is a direct consequence of imperfect task design. Although at least some participants showed a discretisation of phase angles, the phase bins were not wide enough to presume that a phase effect on performance was simply averaged out. It is possible that the auditory and visual systems respond differentially to tACS, although this is not parsimonious under the assumption of neural entrainment as the primary modus operandi of tACS. Another explanation is based on the observation that the electric field strength in tES is, among other factors, influenced by skull thickness and composition (Opitz, Paulus, Will, Antunes, & Thielscher, 2015). The temporal bone, which underlies electrodes targeting the auditory cortex, is generally thinner than the parietal and/or occipital bones which overlie visual cortical areas (Moreira-Gonzalez, Papay, & Zins, 2006), and might therefore be easier to penetrate by the electrical current. No assessment of "phase smearing" as a function of head tissue has to my knowledge been attempted but might be a determinant for a faithful transmission of phase information. However, Helfrich et al (2014) found a phase-modulation of detection accuracy of (central) brief stimuli with 10 Hz-tACS applied through a Cz/Oz montage, targeting occipital cortex. Possibly, this discrepancy arises because of the different target area relative to (similar) electrode position. The montage in this experiment (C2/4 and O2/PO8) was chosen to target the parietal cortex situated between those

two sites, rather than the cortical regions directly underlying either electrode. Modelling work suggests that the maximum current density within such a setup is concentrated between the electrodes (Faria et al., 2012). However, due to the intrinsic anti-phasic nature of a two-electrode montage, the phase reversal may not be as pronounced (as positive and negative polarities at opposite ends cancel out). In such a scenario, phase entrainment by tACS would be predominantly confined to the area immediately below the electrode. This question has implications for the correct choice of montage depending on experimental aims and needs to be addressed using computational models and invasive recordings.

Limitations

There are a number of limitations that need to be addressed in a follow up experiment.

The titration procedure was not very successful in some participants, with often differing thresholds for left- and right-sided targets. Some participants also showed improvement in accuracy over training blocks, making it necessary to lower the threshold further. In several cases these problems lead to floor/ceiling performance where subtle electrophysiological changes would be unlikely to lead to meaningful changes in behaviour. Therefore, inclusion of participants with null effects through inadequate stimulus adjustment could have masked stronger differences between conditions in participants where titration yielded satisfactory threshold estimates. Exclusion of participants with very high or very low performance can help tip the statistical tests over the significance threshold if one so desires but does not change the result that alpha and gamma tACS appear to produce similar deficits. Most participants showed a bias toward one (mostly the right) hemifield. This bias may have been strong enough to override any subtle tACS modulation. This is particularly problematic for bilateral targets and might explain the lack of a response shift as observed by Hilgetag and colleagues (Hilgetag et al., 2001). To improve the design, titration should explicitly address the thresholds for each hemifield separately and include more trials for smoother titration curves.

Individual alpha frequency was estimated from the average power spectrum during task performance. For simplicity, these spectra were not event-

related, i.e., had no systematic relationship to target onset. It could be helpful to calculate power changes relative to the stimulus to get a more accurate representation of task-relevant IAF.

This design did not employ a control montage. Ideally, we would test a mirror-version over the left hemisphere. As there is evidence for cortical asymmetry in visuospatial attention (Capotosto et al., 2012; the most striking one the greater prevalence of neglect after right hemispheric stroke) we cannot automatically assume that a left-hemispheric montage would produce similar deficits in the opposite hemifield. Based on evidence in the TMS-literature, a differential effect for left- and right-hemispheric parietal montages would support the notion that tACS can produce regionally specific interference with spatial processing.

As one of the electrodes was centered roughly over the right primary motor cortex, it could be argued that any effect of tACS in this study could have been caused by interference with the motor response. However, as all but one participant were right-handed, and reaction times were unaffected, this seems unlikely.

Different frequencies may produce differential sensory outcome. The rating of peripheral sensations during breaks between blocks lumped participants' perception over all three trial types. Quite possibly, this is one of the situations in which the average rating does not reflect the actual level of perception within each class of trials. To keep the duration of the experiment short it was decided to collect an aggregate measure of overall sensation rather than a trial-by-trial rating. However, the latter should be considered to get a more accurate estimate of sensory interference over time.

Reports of peripheral sensations were generally low but it cannot be excluded that the observed deficit was a consequence of greater distraction compared to no tACS trials, or through visual interference by retinal stimulation. Some participants also reported a feeling of pressure behind their eyes. Only with below-threshold stimulation (i.e., without any perceptible tACS sensations) could one be confident that any observed changes are indeed of cortical nature,

however such designs may not have sufficient stimulation intensity to reach their neural target (Underwood, 2016).

It is possible that the control frequency was ill-chosen. With respect to Laczó et al.'s findings (Laczó et al., 2012) of frequency-specificity within the γ-band, it would be interesting to test whether a 60 Hz control frequency would have the hypothesised positive effect on stimulus detection. A higher gamma target frequency is also consistent with MEG data showing enhanced power at frequencies above 50 Hz immediately following stimulus presentation (Medendorp et al., 2007; Siegel, Donner, Oostenveld, Fries, & Engel, 2007, 2008). Suppressed (rather than enhanced) γ-activity has also been found during the anticipatory interval (Siegel et al., 2008); if γ-tACS interfered with γ-desynchronisation before target onset (as compared to synchronisation during early stimulus processing) it might have contributed to the observed decline in detection performance.

Conclusion

This experiment has been the second attempt following Brignani and colleagues' example to induce a visuospatial attentional bias by explicitly entraining lateralised α-activity. The preliminary results fail to provide support for the idea that tACS can be used to this effect. At most, tACS may have produced a weak, both frequency- and phase unspecific deficit that might be more related to a disruption of efficient neural processing by interference with a delicate electrochemical balance than to a controlled modulation of endogenous oscillations. However, a number of design issues should be addressed in order to draw more definite conclusions.

Chapter 5. General discussion

Transcranial alternating current stimulation has the potential to be an inexpensive, easily administrable, and well-tolerated multi-purpose tool. Theoretically, it can be applied to establish the functional role of rhythmic brain activity, and to treat neural disorders, in particular those where these rhythms have gone awry. If the potential of tACS is to be harnessed effectively to alter brain activity in a desired manner, it is fundamental to have a good understanding of both the effects of tACS on neuronal dynamics, and of the conditions that are most conducive to produce these effects. To this end, three experiments were conducted to elucidate the mechanism by which tACS interacts with underlying neural network activity. The first two (Chapters 2 and 3), which employed different intermittent α-tACS protocols, were specifically designed to look at post-stimulation aftereffects on posterior α-activity, and how the latter could be explained by prolonged entrainment echoes or spike-timing dependent plasticity. The third experiment (Chapter 4), using an event-related stimulation paradigm at alpha and gamma frequencies, relied explicitly on the assumption of online α-entrainment for its predictions of behavioural changes in a peripheral dot detection task that would reflect the induction of a visuospatial bias through α-power lateralisation.

Overall, the results of these experiments only partially met our hypotheses. Experiment 1 produced the α-enhancement that was expected based on the literature. The lack of entrainment characteristics of this effect suggested that tACS is more likely to induce lasting changes in oscillatory activity by triggering some form of network plasticity. However, the follow-up experiment failed to reproduce these results under similar conditions. This outcome demonstrates at best that tACS aftereffects on α-activity are not robust, may vary widely across individuals, and might be extremely sensitive to small changes in experimental parameters and state variables (although as discussed in Chapter 3, analytical concerns can also not be discounted). The third experiment revealed at most a weak (although arguably spatially specific) impact on target detection that was independent of tACS frequency or the phase of the tACS waveform, which calls into question the assumption of online entrainment as basis for this effect.

Variability of response to tACS

While the limitations pertaining to each experiment were discussed in their corresponding chapters, there are overarching issues that affect these experiments specifically but also tES/tACS research more generally, and contribute to the uncertain outcome of tACS experiments. These are discussed in the following section.

The many degrees of freedom in parameter choice

Mixed results are the rule rather than the exception in tES research (Fertonani & Miniussi, 2016). Realistically, this might be expected in any relatively new area of research where standards have not been set yet and where the boundary between effective and ineffective interventions still needs to be established. This is especially true if the intervention involves many degrees of freedom in the choice of parameters. Indisputably, the parameter space for possible tACS experiments is huge, involving electrode sites, numbers, size, orientation, and shape; the intensity, frequency, and waveform of the current and whether it is applied continuously or intermittently; and whether stimulation is delivered online or offline with regard to the dependent variable. Unlike TMS, which can induce currents that are strong enough to elicit action potentials and produce observable behavioural changes, tACS also does not elicit any (known) immediate, objectively measurable effects by which one could judge the efficacy of a chosen parameter set.

In this light, it is possible that our particular choice of parameters was simply suboptimal for the type of intervention we wanted to test. It is likely that slightly different design decisions could have yielded quantitatively, or even qualitatively, different results. Notably, even moderate changes between Experiment 1 and 2 led to different outcomes (also see the discussion in Chapter 3). The most straightforward way to ascertain how a stimulation effect covaries with a range of parameter values is to systematically test the relationship between plausible values and a given dependent variable, all other things being equal. This seems a daunting task: Collectively, the parameter choices that were made in these experiments (motivated by the extant literature), and those made in previous studies by other groups, cover only a

fraction of the available tACS parameter space. To my knowledge, no systematic effort has yet been started *in vivo* - and specifically in humans - to evaluate the respective contribution of each parameter value *ceteris paribus*. However, computer simulations have been adopted to constrain the parameter space to plausible dimensions, which can then be further reduced by comparing their relative effects on cell slices and live animals (Fröhlich, 2015). Even so, these preliminary measures have to be validated in cognitively active humans.

Choice of a "moving" target

In tACS experiments designed to target a specific neural or cognitive construct, it is often the case that the brain process under study itself is not sufficiently explained, nor isolated from the rest of the brain. In typical human neuroscience experiments, there is a limited focus on specific task situations and brain variables, but even so it is clear that any brain process involves a complex interplay of many areas and frequencies, and that any of these brain variables can vary widely between participants, and can change even within subjects from one trial to the next with behavioural consequences. In other words, the target for stimulation is neither static nor well localised.

Posterior α-activity, the target for tACS in this thesis and the oldest representative of oscillatory brain activity, has been studied for almost 90 years (see Chapter 1, section *The alpha rhythm*). There is an immense amount of data about how and under which circumstances it covaries with a wide number of stimuli, tasks, and mental states. Still, the article alerts remain full of new reports associating α-rhythms with oscillations at other frequencies, new behaviours, or interareal communication. All these findings need to be incorporated into existing theories and indicate that the case "Alpha" is not yet closed. This is especially true for high level cognitive processes and aggravated, ironically, by the just the lack of data showing causality of oscillatory activity.

Moreover, the choice of oscillatory targets is often based on findings from EEG research. The large-scale endogenous electric field patterns measured by EEG represent the sum of the – recordable - activity of many neurons that is spatially low-pass filtered by the skull (Nunez & Srinivasan, 2006), and their characteristics might depend on the level of observation. For instance, the

spatial distribution of the α-rhythm can look very different depending on whether it is recorded from the scalp or from the dura, where more local patterns of synchrony can be observed (Nunez et al., 2001; Perez-Borja, Chatrian, Tyce, & Rivers, 1962). As the observed degree of synchrony may depend on the spatial scale, it begs the question as to how beneficial or detrimental local differences in synchrony are for the process under investigation. In contrast to TMS, which (neglecting indirect network effects) can have a spatial specificity in the range of a few millimetres (Walsh & Cowey, 2000), stimulation is diffuse in tACS, subject to volume conduction, and at least to some degree always involves complementary stimulation at the return electrode. Knowledge about the relevance of local versus global synchrony would be directly relevant in the choice of montage (e.g., high definition/ HD-tACS versus less focal electrode configurations;(Edwards et al., 2013; Helfrich, Knepper, et al., 2014) and affect the interpretation of any changes in behaviour in terms of promotion or disruption of endogenous rhythms. However, such information is not usually available.

As a consequence of the uncertainties about the target, hypotheses cannot incorporate the whole complexity of the system but are practically constrained to limited aspects of neural activity and/or cognition, and assume effects at a specific level of observation. Taking the attempted modulation of spatial attention by enhancing α-activity in one hemisphere as example (Chapter 4), I assumed first, that the chosen tACS montage would induce large-scale, diffuse α-oscillations, and that second, this diffuse α-power enhancement would be both necessary and sufficient to promote an attentional shift. In retrospect, this model is likely to have been overly simplistic. This makes the interpretation of a negative or unpredicted result very difficult.

Inter-and intra-individual variability

Individual differences present an additional source of heterogeneity in non-invasive brain stimulation outcomes (Chew, Ho, & Loo, 2015; Datta, Truong, Minhas, Parra, & Bikson, 2012; Hinder et al., 2014; Labruna et al., 2011; Li, Uehara, & Hanakawa, 2015; López-Alonso, Cheeran, Río-Rodríguez, & Fernández-del-Olmo, 2014; Müller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008; Ziemann & Siebner, 2015). Accordingly, the effect produced by any combination

of parameters (which might *itself* be manifest only in one of any of a large number of behavioural or physiological measures) appears to critically depend on the innate and/or immediate brain state of the stimulated person, including structural and functional anatomy, neurochemical make-up, arousal level and mood, the task a person performs, the level of skill or motivation at which this task is performed, and many other factors that vary between individuals but also within individuals across different occasions or even within sessions.

As a growing body of literature suggests that the state of NIBS is at least to some extent state-dependent (e.g., Benwell et al., 2015; Feurra et al., 2013; Gill, Shah-Basak, & Hamilton, 2015; Neuling et al., 2013; Schutter & Hortensius, 2011; Silvanto, Muggleton, & Walsh, 2008), it is possible that the diverging results of Experiments 1 and 2 were partly a function of systematic group differences in brain state. Indeed, the first sample consisted largely of researchers and postgraduate students at this department who, while unaware of the protocols, conditions, or hypotheses, are highly trained in the performance of cognitive neuroscience experiments and tasks, have intrinsic motivation to produce good data, and can be expected to follow the instructions (such as to maintain covert attention to the disk, which is not strictly necessary to detect its changing colour). The second sample was a more diverse mixture of undergraduate students and non-academic professionals, and may have spent their time on task rather individually. This is of course purely speculative. The point is that even if the experimental parameters can be controlled meticulously, the exact state of participants probably cannot. It is debatable whether it would be advantageous to have a selected group of highly trained and motivated volunteers for a (hypothetically) greater chance of a controlled mental state, or whether participants should be more representative of the general population but with a lower degree of compliance. In the context of a cognitive experiment, the former might be permissible to increase the signal to noise ratio in favour of the neural process under study. However, if the aim of the application is to make generalisable claims about the effect of stimulation, or to test its clinical usefulness, the latter should be one's sample of choice even though such a sample is likely to produce noisier results.

Where do we stand?

With all these challenges, it is not very surprising that the comparatively few existing experimental findings do not supply a complete and harmonious picture. Although tES has been somewhat famed for its ease of use, it becomes increasingly clear that this ease only applies to the physical setting up of the equipment but not to the design choices (Fertonani & Miniussi, 2016), as superficially similar protocols can result in very different outcomes. As an example, in a recent review (Veniero et al., 2015) we summarised some of the findings with regard to the EEG/MEG aftereffects of tACS, amplitude-modulated tDCS, and rhythmic TMS in studies attempting to control certain oscillatory brain dynamics. In brief, we found that while aftereffects after periodic electrical stimulation are frequently reported, their specific nature is far from predictable. Stimulation at specific frequencies has variably been observed to affect spectral activity at the same and/or different frequencies. These spectral effects can be either quite broadband or confined to a narrow frequency range; can result in independent changes of power or coherence; and may or may not be accompanied by associated behavioural aftereffects. Both neural and behavioural changes may be present after stimulation during one "brain state" but absent or different for another (e.g., after stimulation with eyes open or closed, Neuling et al., 2013). For aftereffects at least, it appears to be difficult to control specific neural rhythms effectively by simply adjusting the frequency of stimulation. The reason for this could be in any of the multidimensional parameter space defined by technical and individual factors. Thus, at least for offline protocols, which have the potential to produce lasting beneficial health effects, it is clear that more work must be done to describe the relationship between stimulation and outcome.

Publication bias – Is the efficacy of tACS overrated?

This piece of work is situated in the midst of much bigger controversies in science, and from this vantage point there are several issues worth considering. First, on a more specific level, there are researchers who question the reliability of the reported effects and are at least critical of the usefulness of tES as a research tool (see e.g., Harvey & Kerkhoff, 2015; Horvath, Carter, & Forte, 2014; Horvath, Forte, & Carter, 2014, 2015; Parkin, Ekhtiari, & Walsh, 2015). For

instance, two meta-analyses by Horvath and colleagues concluded that the only reliable tDCS effect can be found for changes in MEPs induced by motor cortical stimulation (Horvath, Forte, et al., 2014, 2015) (a statement which has subsequently been rectified by their failure to find systematic MEP changes across repeated sessions; Horvath, Vogrin, Carter, Cook, & Forte, 2016). Their findings were criticised based on methodological grounds (Antal, Keeser, Priori, Padberg, & Nitsche, 2015; Nitsche, Bikson, & Bestmann, 2015; Price & Hamilton, 2015, see also Horvath, 2015), but as more and more negative findings are published (e.g., Conley et al., 2015; de Hollander et al., 2016; Horvath, Carter, & Forte, 2016; Horvath, Vogrin, Carter, Cook, & Forte, 2015; Tremblay et al., 2016) their scepticism has at least sparked extensive debate among tES researchers (see, for instance, Fertonani & Miniussi, 2016; Harvey & Kerkhoff, 2015; Parkin et al., 2015; Underwood, 2016). While this debate has focused predominantly on tDCS simply because the latter has been around for much longer, and the number of published experiments is much higher, I think it is permissible to assume that the concerns of heterogeneous and poorly replicated results apply equally to tACS.

This local uncertainty about the effects of tES is set in a much wider context sometimes referred to as the "reproducibility crisis", which refers to the finding that a large number of experimental observations across the sciences cannot be replicated at all or only with much weaker effect sizes (Baker, 2016; Boekel et al., 2015; Ioannidis, 2014; Open Science Collaboration, 2015). This problem at least partly reflects political and economic factors in academia that promote a publication bias with an overemphasis on isolated positive findings and lack of information on null results (Ioannidis, Munafò, Fusar-Poli, Nosek, & David, 2014; Pashler & Harris, 2012).

While the tACS literature abounds with interesting observations, these are usually based on small samples and there are very few direct, independent replications. Consequently this should be the focus of future studies as a matter of urgency. A recent meta-analysis of studies employing tACS to induce cognitive changes addressed directly the possibility that publication bias can explain the apparent existence of systematic tACS effects. Schutter and Wischnewski (Schutter & Wischnewski, 2016) concluded based on their meta-analysis of 51

tACS experiments in 24 publications that tACS is effective in producing a small but reliable perceptual and/or cognitive effect. In addition, they calculated the "fail-safe number", the number of non-significant or missing experiments needed to render the result no different from chance. In their estimate, it would require 391 file drawer studies or null findings to wipe out this effect. If the original hypothesis on tACS-induced decline or improvement was taken into account, this fail-safe number increased to 1031 studies. The authors concluded therefore that publication bias at least for cognitive/perceptive studies is unlikely.

Irrespective of the possibility that hypotheses can be adjusted post hoc to fit the data and to improve the narrative to increase the chance of subsequent publication, and notwithstanding the fact that the confidence intervals of the effect sizes of at least 42 of those 51 experiments included zero; this statistical argument is only valid if one accepts (under the assumption those missing and null studies existed) that a success rate of as low as 11% would still be a satisfactory outcome for an intervention that is expected to give clear-cut theoretical insights. Conversely, if for example 11 out of 100 patients with otherwise treatment-resistant schizophrenia could be helped we could speak of an acceptable success rate. Ultimately, tACS could thus turn out to be useful for some of its envisioned application but inappropriate for others. It is important that researchers share information on interventions that worked, but also those that did not work, in order to delineate the utility of ACS for its intended purposes.

Recommendations: How should we proceed

In this section, I will give some recommendations some of which, in hindsight, would have improved the presented studies as well as others in the current knowledge base, and should facilitate the evaluation of published research.

Appropriate choice of control conditions

To be able to assess whether a tACS protocol exerts a systematic effect, an experimental design should incorporate at least a robust pre-stimulation baseline and a control protocol (usually active sham). Several experiments to

date have drawn conclusions solely based on between group comparisons (e.g., Jaušovec & Jaušovec, 2014; Jaušovec, Jaušovec, & Pahor, 2014; Meiron & Lavidor, 2014; Sela et al., 2012) or on a single stimulation protocol (e.g., Neuling, Rach, et al., 2012). Between-group comparisons may be required to assess the efficacy of tACS as a clinical tool in randomised controlled trials but are generally difficult to interpret when there is high inter-individual variability and a low signal to noise ratio in the dependent variable. For this reason, within subject comparisons are indispensable for tES experiments. This applies especially to experiments involving neuroimaging or high level cognitive variables that are inherently noisy.

Between subject experiments also require more participants to achieve similar power to reliably find an effect. It has been estimated that to reliably detect a quite obvious effect, such as that men weigh more than women, one needs at least a sample of 46 *per group* (Simmons, Nelson, & Simonsohn, 2013) – many more than the typical sample size in between subject tACS experiments. As the effects in question are generally quite small, and baseline differences in neural measures and task performance standard rather that exception, it is more economical and facilitates interpretation to test participants repeatedly. This being said, also within-subject studies should include a large number of volunteers, especially if one plans to analyse subgroups for inter-individual differences in mixed designs to assess different response patterns.

Appropriate baseline choice

Within subject comparisons may also present issues if relative changes are compared against a baseline that varies between testing sessions. In Experiments 1 and 2, the variability of α-power at baseline (pre-test) complicated the comparison of relative α-power changes between sessions. This demonstrates the need to sufficiently quantify the baseline variability of the dependent variable in order to evaluate whether changes in this variable are induced by a tES intervention or simply reflect random fluctuations. Note also that if the baseline measurement as such is noisy, it may actually lower the power to find a small effect using change scores, as the noise of the baseline is added to the noise of the post-treatment score. This does not apply if the baseline is controlled for, such as in an analysis of covariance (Simonsohn, 2015). With

multiple repeated measures and baselines, which are common in tACS designs, this is not always straightforward and requires the use of more complex statistical models than required for our familiar t-tests and ANOVAs, such as linear mixed effect models. It might be necessary to run several baseline sessions to be able to differentiate between spontaneous variability and a genuine response to tACS. Increasing trial numbers can help reduce the noise, in particular if trial data are summarised in a meaningful way. The latter point seems trivial, but cannot always be evaluated in the literature (for example, for the distribution of α-power estimates across trials, see discussion in Chapter 3).

In the context of baseline comparisons, it is also important to be wary of derived measures. Ratios or difference scores relative to a baseline can be helpful and valid, but their validity has to be carefully assessed, in particular in the absence of a normalised baseline measure such as MEPs in the study of motor excitability. As Experiment 2 showed, spurious differences can and will be propagated in downstream analyses. The information required to make such an assessment should be provided at a minimum in supplementary materials.

Replicate, replicate, replicate

Even for within subject comparisons, we should not jump to conclusions regarding a potential effect of tES. In any new design, it is both tempting and informative to explore the data in a variety of ways. If preliminary data support a (predicted or surprising) successful intervention, the experiment should be replicated in an independent sample with otherwise identical methods to ensure the effect is not specific to the initial sample. If the original sample size is sufficiently robust, one could analyse one half and use the other as validation group. An even stronger indicator for a genuine effect would be a replication in one or more independent laboratories, as it is done to validate fMRI and MEG measurements. To my knowledge, there are few tACS replications by different groups, in particular no systematic endeavours using identical methods and sample demographics. This limits generalisability of individual experimental findings severely and is in my opinion one of the most urgent issues that need to be addressed.

Replicability of an effect within a person could also be an explicit part of the experimental design. Although it is common to show differences across repeated measures with *different* protocols (for instance verum tACS versus sham), showing a reliable effect of the *same* protocol on multiple occasions is rarely done. A demonstration that the same intervention shows similar or even evolving effects throughout repeated measurements would strengthen the conclusive power of an experiment. This is particularly relevant for potential therapeutic applications.

Mapping the tACS parameter landscape

Regarding the systematic mapping of the huge parameter space, it is informative whether observed effects are specific to a particular set of parameters or even NIBS method, or whether they reflect a general response to stimulation. For example, any observed changes in oscillatory activity or associated behaviour in an experiment should be tested for frequency-specificity. Non-rhythmic stimulation (such as tDCS) or TMS could produce the same effects by mechanisms that do not engage rhythmic network activity directly. For example, as discussed in Chapter 2 and reviewed in (Veniero et al., 2015), α-power can be enhanced after slow frequency stimulation with tSOS, after tDCS (by definition a frequency of 0 Hz), or after TMS at different frequencies. Cross-method validation, as much as control frequencies, are therefore critical but not widely employed. This information is relevant as it informs the mechanism by which any protocol affects changes in the brain.

Another aspect to consider is dose-dependency, for example current intensity. tACS effects have been reported for relatively high (e.g., Pahor & Jaušovec, 2014: >2 mA/pp) and very low intensities (e.g., Voss et al., 2014: 250 μA), and as in my experiments, intensities are often adjusted to match the volunteer's comfort and phosphene thresholds. This approach is common practice in TMS studies where somewhat more objective measures available such as motor threshold are available. However, the possibility of non-linear effects of current intensity (Moliadze et al., 2012) may likely contribute to inter-subject variability in outcome.

As is already standard practice in TMS experiments, tACS experiments should routinely include control montages. A reasonable choice of target and control montages can at this point only be informed by simulations of current flow in the brain (Neuling, Wagner, et al., 2012; Opitz et al., 2016). Such simulations may allow us to choose control montages with minimal overlap in current density distribution. Ideally, these simulations should be calculated based on individual anatomical data using detailed head models and include electrode characteristics. However, fMRI data suggest that electric field models might not be strong predictors of regional metabolic changes in response to tACS (Cabral-Calderin, Williams, et al., 2016). Therefore, validation of simulation-based electric field models in actual human heads is required. With current technologies this is only possible in pre-surgical patients (Opitz et al., 2016) and post mortem brains (Underwood, 2016). Validation can also be sought in animal models, specifically non-human primates or other species with a gyrencephalic brain (e.g., ferrets; Ali et al., 2013). While such data is likely different from healthy human brains, it is the next best step to assess that the current models provide accurate results for a variety of montages.

An appointed time for everything: Feedback stimulation protocols

To date, many tACS experiments apply standard protocols to all their research participants with predetermined time course and frequencies. This is reasonable as it facilitates the comparability of effects across participants or test days. However, it might not be the most efficient way to engage ongoing brain activity and, by extension, induce subsequent changes in neuronal dynamics. Two convincing demonstrations of successful tACS applications have taken advantage of ongoing physiological signals to inform the temporal evolution of their stimulation. In the first, Brittain and colleagues monitored the time course of Parkinsonian tremor during stimulation at tremor frequency and adjusted the phase of their tACS waveform online to produce a favourable phase offset that reduced tremor amplitude by up to 50% (Brittain et al., 2013). In the second example, Lustenberger and co-workers triggered short bursts of tACS at spindle frequency when the ongoing EEG showed characteristic sleep spindles (Lustenberger et al., 2016). This intervention was associated with enhanced sleep spindle activity after stimulation and better consolidation of a motor task. Especially for tACS applications intended to produce clinical or neuro-enhancing

benefits, such feedback stimulation that responds to specific features in the stimulated person's ongoing physiology might be more suitable than rigid designs. It is possible that the α-enhancement in Experiments 1 and 2 would have been more pronounced if the α-tACS trains had coincided with naturally occurring α-spindles, thereby amplifying ongoing brain dynamics rather than trying to impose them. Similarly, it would be interesting to know whether the effect of tACS on visual perception in Experiment 3 was facilitated or prevented by the current rhythmic state. This can be verified experimentally and also underlines the next point – that both neural and behavioural data are required to evaluate a stimulation effect.

Converging evidence from brains and behaviour

The putative α-enhancement described in Experiment 1 is interesting from a theoretical viewpoint but needs to be contextualised with regard to "real-world" practical applications of this technique, i.e., it needs to be shown that this neural change can affect behaviour in a meaningful way. Conversely, the drop in visual detection performance in Experiment 3 is hard to interpret in the absence of neural data. For the sake of interpretability, changes in oscillatory activity must be evaluated by their covariation with associated behaviours and vice versa. While for online EEG experiments this is (to date) largely prevented by artefacts, this should at least be done routinely for aftereffect analysis. Other neuroimaging methods including MEG (Witkowski et al., 2016) and fMRI (Antal et al., 2014) might be less affected by artefacts and should fully capitalise on this opportunity. While the point still applies that there is substantial uncertainty both about effective protocols and the relationship between oscillations and behaviour, showing a link between neural and performance aftereffects will strengthen any interpretation immensely. It must also not be forgotten that with the ability to induce changes in the brain there is a risk of harming people. More inclusive designs are more likely to reveal both benefits and costs of stimulation.

Open science

As for other areas of research, pre-registration of the design including the hypothesis, analyses, and the predicted outcome should be encouraged. The

much-evoked uncertainty regarding the mechanism of tACS as well as the role of oscillations for cognition and behaviour make it hard to predict the outcome of a given intervention but easy to invent post-hoc narratives that can explain any potential observation and might obscure the reasoning behind original design choices. Across the literature, tACS is sometimes interpreted to facilitate, and sometimes to interfere with, the targeted oscillations, while tACS-mediated interference with one mechanism might well result *indirectly* in facilitation of a different mechanism. In addition, often individual differences are often invoked to explain deviations across participants. Electrophysiological data in particular allow for a large range of exploratory analyses with an inflated chance to find significant effects. Pre-registration is one way to safeguard against excessive narrative liberty while still allowing for speculation.

Similarly, transparency as to *all* analyses and results that were performed but not included would be at least as informative as the publication of null results. This would inform other researchers about failed interventions and thereby save time and resources, and allow the development of a more balanced distribution of results that to date is likely to be skewed towards successful attempts but might not necessarily reflect the experience of individual researchers. Openly available data and analysis codes allow others to evaluate putative effects and discover mistakes, and discourage selective reporting, thereby opening an opportunity for more productive science.

Conclusion

A common attribute found in many introductions of research articles involving tACS is "promising" – defined as "showing signs of future success". Such signs, however, have been mainly provided by simulations, in vitro work, and invasive animal recordings. In human neuroscience, compelling evidence for systematic effects remains anecdotal and rarely replicated, especially not by independent laboratories. While tACS may have the potential to be used successfully as a therapeutic or research tool at some point in the future, at present this potential has not yet been realised. Although there is evidence that tACS can produce transient or even lasting changes in the normal neural environment, the field is not yet ready to fully capitalise on these changes in a controlled manner.

The present collection of experiments cannot inform us whether that promise will be kept or broken. Overall, their results paint an ambiguous picture and do not permit confident conclusions about either the mechanisms by which tACS induces its putative effects, or about the causal role of the targeted brain processes. At the most, they allow me to argue weakly against frequency-specific phase entrainment under the specific test conditions but lack explanatory power regarding alternative mechanisms. Nonetheless, despite all associated limitations, the described effects could be suggestive of a genuine interaction between tACS and neural activity that could be harnessed more effectively and reliably through methodological fine-tuning. The interpretational difficulties in these experiments do highlight a number of problems that affect studies involving tES in general and tACS in particular, including their design, analysis, and interpretation, and inspire guidelines towards more informative and replicable research designs. Careful experimental procedures combined with openness about both null results and violated expectations, and perseverance in the systematic exploration of a vast parameter space from converging research angles, make it more likely that we learn about this technique's true potential, and to disentangle hopes that are hype from those that are real possibilities.

Appendices

Appendix A: Screening questions in tACS safety questionnaire

Have you ever:

- Had tACS before? (Yes/No)
- Had an adverse reaction to tACS? (Yes/No)
- Had a seizure? (Yes/No)
- Had an unexplained spell of loss of consciousness? (Yes/No)
- Had any brain-related, neurological injury or illnesses? (Yes/No)
- Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips, cochlear implant or fragments from welding? (Yes/No)
- Do you have any implanted medical devices such as cardiac pacemakers, or medical pumps? (Yes/No)
- Do you suffer from frequent or severe headaches? (Yes/No)
- Are you taking any medications? (Yes/No)
- Have you recently taken any recreational drug or alcohol? (Yes/No)
- Are you sleep deprived? (Yes/No)
- Are you pregnant, or are you sexually active and not sure whether you might be pregnant? (Yes/No)
- Does anyone in your family have epilepsy? (Yes/No)
- Do you need any further explanation of tACS or its associated risks? (Yes/No)

FOR ANY « YES » RESPONSE, PLEASE PROVIDE DETAILED INFORMATION

Appendix B: Post-session questionnaire (Exp. 1 and 2)

This questionnaire refers to your experience of the stimulation during the visual task.

How strongly did you perceive a tingling, itching, or burning during the stimulation? Please draw a vertical line[2] below to indicate how strongly you felt either sensation.

no tingling/ itching/ burning ⟝——————————⟞ very strong tingling/ itching/ burning

If you experienced a tingling/itching/burning sensation, did you experience it (please circle)

Rarely occasionally continuously in the beginning only?
More under left electrode more under right electrode on both sides equally?

How unpleasant was the stimulation for you? Please draw a vertical line below to indicate as how unpleasant you experienced the stimulation.

did not notice at all ⟝——————————⟞ painful

If you experienced the stimulation as unpleasant or painful, did this occur (please circle)

Rarely occasionally continuously in the beginning only?

How strongly did you experience flickering or light patches in your visual field independent of the colour stimulus you had to attend to? Please draw a vertical line below to indicate how strongly you perceived visual side effects during stimulation.

no flickering/ light patches ⟝——————————⟞ vivid flickering/ light patches

If you experienced flickering or light patches, did you experience them (please circle)

Rarely occasionally continuously in the beginning only?
Centrally more in left visual field more in right visual field in both fields equally?

Can you draw a shape?

Are you experiencing any discomfort now? If so, what and how severely?

[2] VAS lines were 10 cm long.

Appendix C: Rating of peripheral sensations (Experiment 3)

- How much do you feel the stimulation on your skin?
- How much do you experience discomfort?
- How much do you experience visual disturbances (flickering/flashing/wobbling/...)?

ANSWER FROM 1 = not at all TO 7 = very strongly

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