Do somatosensory oscillations relate to tactile attention?

Extracting the phase of transcranial Alternating Current Stimulation (tACS) during stimulus presentation.

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Abstract

Attentional mechanisms allow for the prioritization of information depending on the task at hand. Evidence from Electroencephalography (EEG) suggests that lateralised changes in the amplitude of alpha oscillations (8-14 Hz) are linked to orienting attention and that the phase of an oscillatory cycle can affect how behavioral and perceptual information is processed. Transcranial alternating current stimulation (tACS) is a non-invasive brain stimulation method that involves the application of weak electric currents to the scalp. tACS provides the ability to entrain intrinsic oscillations to specific frequencies. Through the employment of new hardware, the timings of stimuli presentation and the phase of tACS signals were accurately recorded so that their timings could be compared. This setup was implemented in an ongoing study that utilised participant individualized alpha and beta (25 Hz) stimulation during two tactile attention tasks. Results indicated that during alpha stimulation, performance in an endogenous tactile attention was mediated by the phase of the tACS signal, with a distribution of reaction times (RTs) that approximately followed the pattern of the waveform signal. The phase of the tACS signal during beta stimulation was shown to mediate performance during an exogenous tactile attention task. Both these results indicate that the fastest and slowest RTs occur at opposite phase positions of the tACS signal, providing novel evidence for a phasic relationship between performance variability and somatosensory attention.

Acknowledgments

# Contents

1. Introduction

1.1 Attention

Our sensory system is constantly bombarded with information. In order to successfully interact with the environment and achieve our goals, we need to select and prioritize certain events and stimuli over other. This is generally known as attention (Carrasco & Barbot, 2018). Spatial attention, the process of orienting to a specific location in space, is typically divided into endogenous and exogenous orienting.

Endogenous attention (also known as top-down or voluntary attention) is when the individual voluntarily decides where to shift their focus, such as attending to the road when driving. Whereas exogenous attention (bottom-up, involuntary stimulus-driven attention) is reflexive and mediated by external stimulation. For example, something suddenly jumping out from the side of the road would attract our exogenous attention (for a review, see Carrasco, 2011). Orienting endogenous visual attention to a spatial location has been shown to enhance perceptual processing (e.g., Mangun and Hillyard, 1990; Yeshurun and Carrasco, 1998) as well as improve reaction times (RTs) for stimuli at cued (where the cue indicates the location of the target) compared to uncued (where no target information is available) locations (Carrasco, 2014). Exogenous visual attention demonstrates both faciliatory and inhibitory effects on RTs for cued compared to uncued targets dependent upon the elapsed time between cue and target (Posner & Cohen, 1984). In their experiment Posner and Cohen, instructed participants to fixate on a central box set between 2 boxes at the left and right. During each trial the outline of one of the boxes glowed for 150 ms and provided a spatial cue that exogenously attracted visual attention. After an interval (referred to as a stimulus onset asynchrony; SOA), that varied in length from trial to trial, a target was presented at either the same (cued) or opposite (uncued) position as the cue. They found that the speed that the target was detected at was faster for cued compared to uncued stimuli until the SOA was in excess of 300 ms, at which point an inhibitory effect replaced this facilitation. That is, when the cue and target interval was less than 300 ms then a faster response time was seen when the cue and target appeared at the same position, compared to when they appeared at opposite positions. When the cue and target interval exceeded 300 ms then responses were found to be slower if the target appeared at the same position as the cue, compared to the

opposite position; a phenomenon termed inhibition of return (IOR; Klein, 2000). This facilitation effect is proposed to reflect an initial reflex towards the cue, that allows effective processing of the stimuli and its location (Posner, 1980; Posner & Cohen, 1984; Yantis & Jonides, 1984). The inhibitory effect was branded IOR due to the inhibiting of a return to stimuli recently processed (Posner, Rafal, Choate & Vaughan, 1985). During endogenous orienting, inhibition is not observed and the level of predictability that the cue provides for the target appearance affects the speed of responses. That is, the more predictive the cue is of the upcoming target location, the more efficiently it is processed (Chica & Lupiáñez, 2009; Wright & Richard, 2000).

Though most of the research has been carried out in the visual domain, spatial attention has also been investigated in touch, often using a variations of the visual Posner cue-target paradigm (Posner, 1980). In the endogenous version of the tactile paradigm a visual or tactile cue, such as an arrow or stimulation to the finger, provides spatially relevant information on where to expect a tactile target (see Figure 1). Endogenous attention towards a body location has, similar to vision, been shown to improve RTs for cued targets compared to targets presented at unattended location (Jones and Forster, 2014, Spence and Gallace, 2007). How predictable the cue is of the target has also been shown to effect RTs. 100% predictability has been shown to produce the fastest RTs, 75% predictability further reduces RTs and at 50% (i.e. not predictive) RTs are at their lowest (Haegens, Handel, & Jensen, 2011).
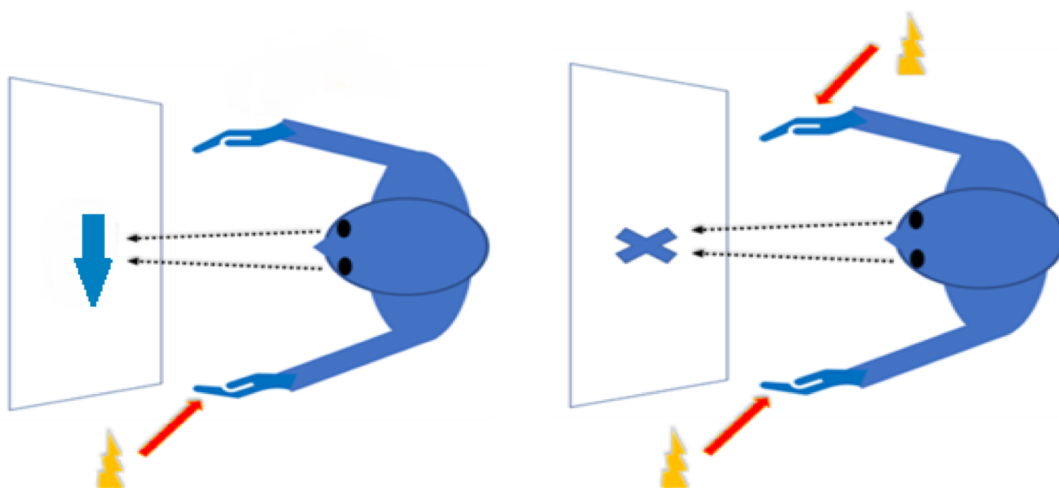
Figure 1. **Left**: Endogenous tactile attention. Knowledge of where to attend is provided and so tactile stimulation is expected at that location. **Right**: Exogenous tactile attention. No information regarding where to expect tactile stimulation is provided and attention is oriented reflexively.

In exogenous tactile attention, the cue provides no information on where a tactile target may appear (see Figure 1) and a similar IOR effect to that seen in vision can be observed (Jones and Forster, 2012, Lloyd et al., 1999). Research contrasting both forms of attention have indicated separate RT effects for visual (Berger, Henik, & Rafal, 2005) and tactile orienting (Jones and Forster, 2013, Jones and Forster, 2014), providing evidence that they are separate mechanisms (see Chica, Martín-Arévalo, Botta & Lupiánez, 2014). Both forms of attention have shown to have distinct neural pathways (Corbetta & Shulman, 2002). Endogenous attention is shown to be influenced via the parietal and superior frontal cortex whereas exogenous attention is mediated via the temporo-parietal and inferior frontal cortex (see Macaluso, 2010 for a review). In tandem with the research of connectivity involved in attentional activity, emerging evidence on the functional role of brain oscillations in attention has also come to the forefront (Calderone, Lakatos, Butler & Castellanos, 2014).

1.2 Alpha oscillations

The electrophysiological activity measured on the surface of the scalp using Electroencephalography (EEG) or Magnetoencephalography (MEG) demonstrates oscillatory activity across different frequency bands. This rhythmic activity is constantly occurring regardless of the level of task being carried out. The bands of rhythmic activity are typically separated into five different frequency ranges with arbitrary and, to a certain degree, variable margins (Başar, Başar-Eroglu, Karakaş, & Schürmann, 2000; Wang, 2010). The five bands take their names from Greek letters: Delta (0 - 4 Hz), theta (4 - 8 Hz), alpha (8 - 14 Hz), beta (14 - 30 Hz), and gamma (greater than 30 Hz). Different researchers may class the ranges slightly differently than the ones stated here, they may be further subdivided (e.g. low alpha, high alpha, low beta etc.) as and when deemed suitably descriptive, and the exact frequency of activity will vary between individuals. In order to classify an oscillation to its respective frequency range, the amount of time taken to complete one cycle is measured from peak to peak (see Figure 2). For example, if a complete oscillation takes 100 ms, then it has a frequency of 10 Hz (occurring 10 times a second) and belongs in the alpha range. For a frequency to be determined the power of the ongoing electrophysiological activity fluctuates over time and the varying amplitude produces a visible wave-like formation demarked by peaks and troughs. The

peak and trough of an oscillation can be considered the simplest representations of phase angles seen in electrophysiological activity. A phase angle can be any specific time point within an oscillation but, due to varying frequencies, is expressed as radians or an angle that corresponds to one complete 360° oscillatory cycle. One important aspect in the measure the ongoing electrophysiological activity is that the amplitude can vary and still produce observable peaks and troughs without altering their phase angles or the frequency of the oscillation. The most visible rhythm in the adult human brain is alpha activity (Klimesch, 2012). While originally considered to reflect cortical idling and often dismissed as a biological artefact, a growing amount of research has consistently linked it to specific functional roles in cognition and behavior.

Although almost 100 years have passed since alpha was first observed by Hans Berger (Berger, 1929). A plethora of research expanding half a century has linked alpha to numerous cognitive processes including, among others, memory (Bonnefond & Jensen, 2013), intelligence (Doppelmayr et al., 2005), oculomotor control (Wertheim, 1974), arousal (Makeig & Jung, 1995) and attention (Thut, Nietzel, Brandt, & Pascual-Leone, 2006; for a comprehensive review of early alpha-rhythm research see Shaw, 2003).

MEG Source localisation of posterior alpha-rhythms indicates that the activity originates from regional neuronal clusters located at the parieto-occipital cortex (Thut, Schyns, & Gross, 2011). These alpha-generators have also been observed in different cortical layers (Bollimunta, Mo, Schroeder, & Ding, 2011). Invasive recordings also indicate that these sources are made up from populations of neurons that are consistently changing between being in and out of synchrony with each other (Nunez, Wingeier, & Silberstein, 2001). When a sufficient group of neuronal clusters oscillate coherently at any one time, their collective amplitude becomes powerful enough to be a visible feature of non-invasive recordings such as MEG and EEG.

Evidence suggests the functional role of alpha-band oscillations in attention, with power within the alpha range shown to be modulated when visual attention is shifted from one area of space to another (Calderone et al., 2014). Moreover, the contemporary view is that endogenously orienting attention to the body leads to a modulation of alpha power in the somatosensory cortex (see Figure 2). When attention is directed to one side of space the contralateral hemispheres demonstrates decreased alpha activity and

the ipsilateral hemisphere shows an increase in alpha power (Jensen & Mazaheri, 2010). This modulation of alpha oscillations is said to reflect neural changes leading to improved processing of sensory information (Ikkai, Dandekar & Curtis, 2016). This proposition is further strengthened by numerous studies showing correlations between improved target detection and decreased alpha power (Gould, Rushworth & Nobre, 2011; Händel, Haarmeier & Jensen, 2011). Similarly, in cross modal studies involving a cue that indicates whether to expect a visual or auditory target, alpha activity shows a relative increase and decrease in both the occipital and auditory cortex (Mazaheri, van Schouwenburg, Dimitrijevic, Denys, Cools & Jensen, 2014; Gomez-Ramirez, Kelly, Molholm, Sehatpour, Schwartz & Foxe, 2011).

Evidence for this alpha lateralization comes from attentional cuing paradigms demonstrating fluctuations in the amplitude of oscillations occur due to top–down control. These changes in local alpha power are now widely considered to be the mechanism whereby attention is directed. An increase in alpha amplitude is equated with the suppression of irrelevant information, whilst an alpha amplitude decrease is seen to occur in areas associated with the processing of relevant information (Foxe & Snyder, 2011; Jensen & Mazaheri, 2010; Klimesch, Sauseng, & Hanslmayr, 2007; Palva & Palva, 2007; Snyder & Foxe, 2010). This relative modulation of alpha is present not only during visual and auditory attention but also when attention is focused on tactile sensations (Bauer et al., 2012; Jones et al., 2010; Haegens et al., 2011; Schubert et al., 2015). Using both an endogenous and exogenous spatial task Haegens and colleagues (2011) explored the role of alpha oscillatory activity using a visual cue that directed attention toward a tactile target that occurred at either the left or right hand. Their results indicated a correlation between the lateralisation of alpha oscillatory activity and performance; with accuracy and RTs showing improvement with the degree of alpha lateralization. The study also provided evidence for a graded lateralisation effect depending on the cue's level of predictability. When the cue was 100% predictive of the target location then the lateralisation of alpha activity was at its highest, at 75% predictability the lateralisation was reduced and at 50% (i.e. not predictive) lateralisation was almost absent. Essentially the study demonstrated differing alpha power changes between endogenous and exogenous attention at somatosensory

occipital regions that are specific to tactile processing and mirror the behaviour of posterior alpha oscillations in visuo-spatial attention. Following these observations further research has shown that the amplitude of alpha oscillations prior to stimuli presentation significantly affects any perceptual outcomes (Kanai, Chaieb, Antal, Walsh, & Paulus, 2008; Romei, Gross & Thut, 2010; van Dijk, Schoffelen, Oostenveld & Jensen, 2008).
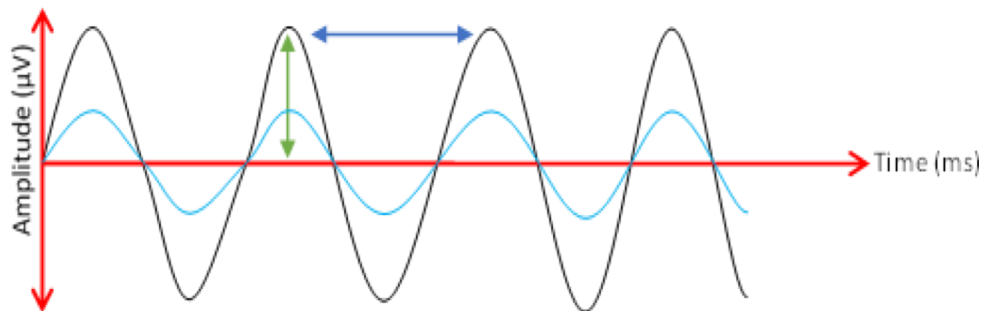
Figure 2. Exemplary EEG components and lateralisation during attentional tasks seen at the somatosensory cortex. Black line represents the alpha waveform (8- 14 Hz) at the hemisphere ipsilateral to attended space. Light blue line represents the alpha waveform at the hemisphere contralateral to attended space. Green arrow indicates higher amplitude relative to baseline at the hemisphere ipsilateral to attended space compared to the hemisphere contralateral to attended space. Blue arrow indicates how frequency is determined by the distance from peak to peak within a single oscillation. The highest point (peak) and lowest point (trough) of each waveform are separated by half an oscillation and represent phase angles 180° apart.

Whilst the general consensus is that an increase in alpha power is related to top-down suppression of distracting information, it is worth noting that relatively little research has fully explored the role of these neuronal processes. There is evidence for benefits of both the suppression of distractors through increased alpha power and the reduction of power to enhance target detection in both the visual (Okazaki, De Weerd, Haegens & Jensen, 2014; Zumer, Scheeringa, Schoffelen Norris & Jensen, 2014) and somatosensory domain (Fu, Foxe, Murray, Higgins, Javitt & Schroeder, 2001). Although there is a lot of evidence supporting alpha's role as an inhibitor of attention, how exactly this is accomplished is still to be determined. The findings in the cognitive domain indicate that the topography of the alpha frequency, its amplitude and interactions with other frequency ranges are instrumental in the global functioning of information processing, rather than solely responsible for a limited number of mental processes (Fries, 2015). Taken together, there is an increasing body of evidence which has observed the effects of cortical alpha amplitude changes in relation to perception and

attention, however alpha oscillations are not purely defined by their amplitude but also their phase.

1.3 Phase

One continually replicated outcome in studies of cognition is an observed variability in performance following continued presentation of stimuli across seemingly identical experimental procedures. This variability often manifests as different levels of perception from trial to trial in perceptual tasks or a wide range of RTs across trials in attentional tasks. A developing consensus in the study of alpha oscillations is that not only the power of oscillatory alpha activity plays an important role in perception but also the phase (Jensen, Gips, Bergmann & Bonnefond, 2014; Klimesch, 2012, Klimesch, Sauseng & Hanslmayr, 2007, Mathewson et al., 2011, Palva and Palva, 2007, VanRullen, 2016a). The phase of electrophysiological activity refers to a specific moment along an oscillatory cycle. In its simplest form this can refer to the peak or trough (see Figure 2), which represent distinct moments within a cycle, with research indicating the greatest variance in perceptual performance is seen between these two phases. These studies (referenced above) suggest a rhythmic component in visual perception, where the phase of alpha oscillations determines whether stimuli are consciously perceived. The ability to detect a near threshold stimuli was found to be dependent on the moment within a single oscillatory cycle that stimuli was presented. This line of research is not new and studies going back 60 years have tested EEG phase and behavioural responses (Callaway and Yeager, 1960, Dustman and Beck, 1965, Varela et al., 1981). Evidence shows that increased detection of visual stimuli is dependent on when in the phase of alpha oscillations presentation occurred (Busch, Dubois & VanRullen, 2009; Dugué, Marque & VanRullen, 2011; Fiebelkorn, Snyder, Mercier, Butler, Molholm & Foxe, 2013; Mathewson, Gratton, Fabiani, Beck & Ro, 2009), with detection performance for attended stimuli decreasing monotonically the further away they were presented from the optimally performing phase angle (i.e. performance differences were maximally separated by half an oscillation). Subsequent studies have addressed alpha phase oscillation relationship with stimulus onset and behavioural outcome in alternative

domains including memory, ERPs, auditory and tactile perception (Gundlach, Müller, Nierhaus, Villringer & Sehm, 2016; see VanRullen, 2016a for a recent review).

Additional evidence using functional magnetic resonance imaging (fMRI) has also demonstrated that the onset of visual stimuli modulated blood oxygenation level-dependent (BOLD) responses in the early visual areas are dependent upon where in the phase of alpha oscillations they were presented (Scheeringa, Mazaheri, Bojak, Norris, Kleinschmidt, 2011). Non-human studies have also provided evidence for the phase specific properties of alpha during a discrimination task (Haegens, Nacher, Luna, Romo & Jensen, 2011). Measuring neuronal activity at the sensorimotor cortex Haegens and colleagues found neuronal spiking to be associated with the alpha phase in local-field potentials, indicating that the oscillatory phase acts as a modulator of neuronal activity. These physiological findings suggest that alpha's inhibitory influence on spontaneous neuronal activity acts in a phase specific manner during an alpha cycle rather than throughout the whole inhibitory period (Mathewson et al., 2011; Mazaheri & Jensen, 2008).

Contemporary researchers have attempted to integrate these physiological and behavioral findings in to an encompassing theory of alpha. The "pulsed inhibition" hypothesis (Mathewson et al., 2009) states that an alpha-oscillation acts as a rhythmic filter that "pulses" between the cyclic inhibitory states of the peak and trough, where populations of neurons oscillate between an excitable or inhibited state. The "gating by inhibition" hypothesis (Jensen & Mazaheri, 2010) proposes that local changes in the power and phase of alpha through endogenous attentional control determine how extensively information is processed at the neuronal level. This proposed framework sees alpha activity acting as a filter that blocks irrelevant information and only allows salient information to be more fully processed. The greater the number of coopted neurons the higher the alpha amplitude is and the stricter the filtering. Similarly, the "inhibition timing" hypothesis (Klimesch et al., 2007) adopted the same fundamental idea, but emphasized the importance of timing and communication between functionally related areas and their associated neuronal networks. This framework essentially highlights that coherently precise communication across the brain allows for effective processing of relevant information, with the power and phase of alpha oscillations representing coordinated interactions between relevant brain regions. When

regions are not actively oscillating together their communication is suppressed allowing for only salient processing. Related to these views is the idea of ongoing cyclic activity as a rhythmic perceptual sampler, where perception is not considered to be a continual process, but rather external information is periodically sampled (Busch & VanRullen, 2010; Schroeder & Lakatos, 2009). In this framework, the exact frequency (albeit alpha or other cortical bands; see Fiebelkorn & Kastner, 2018 for an attentional theory related to the theta rhythm) regulates the sampling rate, and the phase of that frequency determines when information is sampled. In contrast to the hypotheses of inhibition, this theory highlights, not how inhibition blocks distractors, but rather how information flow at discrete moments in an oscillatory cycle are the key component of cognitive processing (Jensen et al., 2014, Klimesch et al., 2007, VanRullen, 2016a).

A common proposal among these theories is that processing occurs (either through the restricting or allowing of information flow) not on a continuum, but at phasic intervals that are cyclic by nature. Empirical observations lend support for rhythmic alpha activity as an inhibitor of irrelevant information that can both alter perceptual thresholds and illicit periodicity from perceptual performance (e.g., Bonnefond & Jensen, 2012; Dugué et al., 2011; Mathewson et al., 2011). Understanding how the power and phase of alpha oscillations and cognitive processing are linked has largely been tackled by measuring EEG or MEG and correlating it with performance using an appropriate paradigm. Various EEG and MEG studies have demonstrated that the power, frequency, and phase of alpha oscillations can reliably predict whether visual stimuli are perceived or not (Busch et al., 2009; Mathewson et al., 2009; Samaha and Postle, 2015).

The analysis of phase dependent perception using EEG can follow a variety of different methodological approaches. For example, Busch et al. (2009) used a visual task involving the orienting of spatial attention to analyze the effects of pre-stimulus phase on perception. Their analysis covered almost the entire spectral frequency (3 Hz – 100 Hz) with a time window ranging from -800 ms to stimulus onset and all EEG electrodes. Busch and colleagues used a combined index called a phase bifurcation index (PBI), which was based on a comparison between a measure of inter-trial coherence (ITC) for hit and miss trials, against the overall ITC for all trials. They found that perception of near threshold stimuli was modulated by the phase of ongoing EEG oscillations at

stimulus onset, with hit and missed trials being associated with different phase angles. In contrast, Mathewson et al. (2009) used a detection task where visual attention was focused centrally. Their choice of paradigm meant phase analysis was focused on a limited pre-stimulus time window. Using a trial-by-trial analysis they sorted behavioral responses in to one of two opposing phase bins, depending on where along an oscillation stimulus occurred. From the two phase bins they were able to determine whether performance differed significantly as a function of phase. Using participants with differing alpha frequencies Samaha and Postle (2015) showed that those with faster individual alpha frequencies were more likely to perceive two independent flashes with identical intervals as one, suggesting that the temporal resolution of perception is related to the exact length of an alpha oscillation. Alternatively, the phase of alpha oscillations at stimulus onset may be compared between conditions with differing attentional requirements. The number of phase bins may extend beyond two, however, to achieve a reliable estimate of phase distribution a large number of trials is required. Also, a significant difference between both conditions can only be reported when both demonstrate a preferred phase-angle. If an effect of phase is expected only for one condition and random in the others, a measure known as Phase Locking Factor (PLF, also known as inter-trial phase-locking; ITPC), is often used (Muthukumaraswamy & Singh, 2011).

PLF refers to the complex average of the phase-angles across trials normalized to a value range of 0 and 1. If the PLF value between areas is close to 0 then this signifies a random alignment of phase between them, whereas a value close to 1 indicates that the phase between them occur in concert. This allows the precise moment along an oscillation to be measured and associated with stimuli presentation. The higher the PLF the more consistently stimuli presentation occurs at a specific phase angle. However, this method is not without limitations. For example, the amplitude of oscillatory activity can influence the PLF due to differences in the signal to noise ratio between conditions. These differences may manifest in the PLF and lead to false positives (see van Diepen & Mazaheri, 2018 for an advanced discussion on the matter). In addition, phase locked evoked responses caused by the onset of stimulus presentation means that any phase locking within a trial is susceptible to temporal leakage from the ERP, especially when

the phase of interest is close to stimulus onset. This means that any changes in the ERPs due to top-down processing (i.e. by expectation or attention) can lead to changes in the PLF regardless of any changes in the phase of ongoing oscillations.

These studies illustrate the basic principles of phase distribution research in perception as well as some differences in the methodological approach and issues with EEG analysis. Subsequent studies have followed these general frameworks with variations in the post-processing methods as well as the scale of frequencies, location, and the timeframe of alpha phase activity under scrutiny. The general underlying logic is that in the frequency of interest the phasic position of pre-stimulus activity from successfully perceived trials should be different to the phase position when trials are not consciously observed (VanRullen, 2016b).

As discussed, understanding how alpha oscillations and cognition are linked has largely been tackled by measuring EEG or MEG correlated with behavioural performance. However, a growing number of researchers have adopted the inclusion of brain stimulation techniques as a method to infer regional roles of neuronal activity in the brain.

1.4 Electrical brain stimulation techniques and tACS

Transcranial direct current stimulation (tDCS), transcranial random noise stimulation (tRNS) and transcranial alternating current stimulation (tACS), are a collective of various transcranial electrical stimulation (tES) techniques (Bikson, Reato, & Rahman, 2013; Woods et al., 2016). These methods act on the stimulated region by the induction of a subthreshold polarization through the scalp to neurons below, that causes a change in neuronal firing rates at the targeted area. This does not lead to the firing of action potentials but rather an alteration to the polarization of the resting membrane potential, such that the likelihood of an action potential occurring can be manipulated. Although tES methods do not directly induce an action potential, they do increase and decrease the probability of an action potential occurring depending on the polarity of the stimulation (Antal & Herrmann, 2016).

These various non-invasive electrical stimulation techniques can be performed using the same hardware, where a weak electrical current (usually less than ±3 mA) is passed between two or more electrodes attached to the surface of the scalp. The spatial

specificity of the electrodes depends upon the type used, but is in the range of centimetres, with additional focality available if smaller electrodes are used or ring montages are adopted around the electrode above the target area. The differences between these stimulation protocols is in their differing electrical waveforms and the neural effects they produce. The general principle is that anodal stimulation leads to an increased resting membrane potential for the underlying neural tissue, whereas the resting membrane potential of neurons at the cathode is lowered (Nitsche & Paulus, 2000). The mechanism behind this modulation is due to changes in the resting potential voltage of the stimulated areas neurons. Although, studies show that polarisation of underlying neurons is dependent on factors such as cell depth or orientation as well as neuronal connectivity between local and global populations (Bikson et al., 2013). Despite this cautionary note, the above description is generally accepted as a reasonable explanation for the mechanisms whereby tES techniques operate (Jacobson, Koslowsky, & Lavidor, 2012).

tDCS is the most commonly used technique for brain stimulation in cognitive and clinical neuroscience research. As indicated by its name, the electrical waveform is direct and does not change over time, i.e. the polarity and intensity are not altered throughout the entire stimulation procedure. Each electrode pair consist of an anode (where current flows inwards) and a cathode (where current flows outwards). tACS and tDCS are similar in their respective applications in that they typically utilize comparable montages and current strengths.

In contrast to tDCS, tACS, as its name suggests, involves an alternating electrical waveform that is set to periodically change direction at a pre-specified frequency, that is, the polarity is alternated between the anode and cathode at a set time creating an oscillation between the electrode sites (see Figure 3). This rhythmical reversal of the flow of electrons by alternating the positive and negative voltages at regular intervals allows for the manipulation of neural oscillations in a frequency specific way (Tavakoli & Yun, 2017). As differing functions are associated with specific cortical frequencies (Thut, Miniussi & Gross, 2012) tACS can more directly influence these functions. The tACS waveform is usually sinusoidal, although waveforms such as box-car or saw-tooth can be used. tRNS also uses an alternating current with the addition of randomly changing amplitudes and frequencies (the effects of which more closely resemble tDCS than tACS;

Chaieb, Paulus, & Antal, 2011). The application of tACS enables the safe and non-invasive modulation of ongoing neural oscillations. Research using tACS has shown to effect behavioural performance related to the neural network or targeted oscillation frequency (Cecere, Rees & Romei, 2015; Wolinski, Cooper, Sauseng, Romei, 2018). There is evidence to suggest that tACS applied during experimental procedures entrains the ongoing oscillatory activity (Veniero, Vossen, Gross & Thut, 2015) and the various parameters that must be adjusted to implement a successful tACS protocol are discussed below.
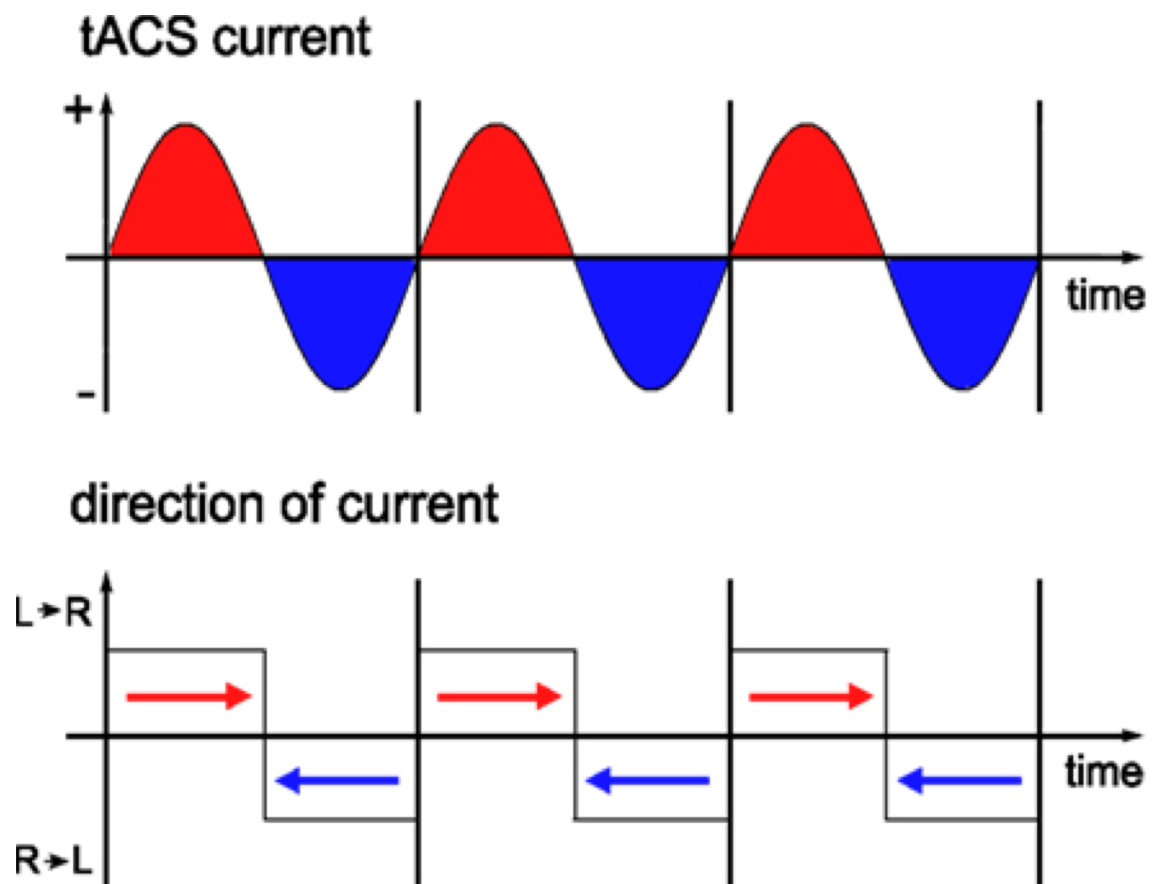
Figure 3. tACS waveform current (top) and direction (bottom). The alternating electrical waveform is set to periodically change direction at a pre-specified frequency. The polarity is alternated between the positive anode (red) and the negative cathode (blue) at a set time, creating an oscillation between the electrode sites that switches the positive and negative voltages at regular intervals. This allows for the manipulation of neural oscillations in a frequency specific way. The two electrode sites are always in anti-phase, where they follow the same frequency, but at opposite phase positions. The highest and lowest point of each waveform (top) are separated by half an oscillation and represent phase angles 180° apart.

The montage refers to the location of electrodes, how many are used and their relative orientation to scalp topography. Electrode positioning can either be at different

places on the scalp (depending on the region of interest) or an extracranial reference can be used, such as on the shoulder. This would reduce the number of active electrodes that directly interface with the scalp. The montage placement is also important for determining the flow of current between electrodes, which in turn effects where maximal stimulation of brain regions will occur (Neuling, Wagner, Wolters, Zaehle, & Herrmann, 2012). An additional concern in placement choice is to what level it may stimulate the retina, inducing phosphenes (Laakso & Hirata, 2013) and to what level it will promote the passing of electrical current through the skin (Faria, Hallett, & Miranda, 2011).

The applied current is usually below 3 mA from peak to trough (peak to peak amplitude), with changing scalp resistance constantly monitored and adjusted for during stimulation. Recent evidence in non-humans suggests a current strength as low as 0.5 mA may be sufficient to stimulate underlying neuronal populations (Johnson et al., 2019). Although conflicting evidence also suggests that very little current may penetrate the scalp and cerebrospinal fluid (Vöröslakos et al., 2018).

Various types of electrodes can be used to apply the electrical stimulation, including rubber electrodes or standard EEG electrodes. To combat impedance between the scalp and electrode they are either attached to sponges immersed in saline solution and held in place by rubber bands or electrode paste is applied which also acts to hold them in place on the head. The smaller the electrode the more focal and the stronger the current density below it. Electrical field modelling indicates that the greatest current density lies at electrodes' edges and directly underneath it, and the further apart electrodes are placed the larger the field strength across the entire cortex (Saturnino, Antunes, & Thielscher, 2015).

One important component of the oscillating tACS signal is the frequency it is set to. The speed at which a full oscillation occurs is usually chosen to match a known cortical frequency (i.e. alpha, beta, delta, gamma, theta) previously associated with a cognitive state or function observed in EEG or MEG recordings. When a montage contains two electrodes stimulation it is said to be in "anti-phase". That is, when the current at one electrode is positive the other electrode will be negative, in tACS this anti-phase relationship is alternated between the pair of electrodes at a fixed frequency. With a more complex montage containing more than two electrodes the phase of each

pair can be timed so that their waveforms are phase aligned, i.e., two electrodes have a positive or negative current at the same time. When this arrangement is used the level of phase alignment between electrodes is considered to affect the coherence between communicating neuronal populations (Helfrich et al., 2014; Polanía, Nitsche, Korman, Batsikadze, & Paulus, 2012).

When a tACS experiment involves stimulus presentation, the moment in an oscillatory cycle that the stimuli are presented is often to referred to as its phase. As with studies using EEG, as a measure of intrinsic oscillations, the phase of tACS induced oscillations have also been linked to the trial by trial variations in perceptual thresholds (e.g., Gundlach et al., 2016; Riecke, Formisano, Herrmann, & Sack, 2015; Romei, Gross, & Thut, 2012; VanRullen, Busch, Drewes, & Dubois, 2011). Studies using Intracranial recordings suggest that very little phase distortion exists between the anodal and cathodal electrodes, such that the overall electrical field can be considered to represent the phase of the ongoing oscillation (Opitz et al., 2016). Based on this evidence the relationship between the spatial distribution of the tACS signals and its phase can be assumed to be stable. The alternating cycle of the tACS current between a pair of electrodes imposes a fixed temporal structure on the underlying neuronal populations due to periodic depolarisation and hyperpolarisation. The increase and decrease of the membrane potential shapes the firing rates of action potentials providing control over neural communication (Fröhlich & McCormick, 2010). The alignment of the tACS frequency to task related intrinsic neural activity provides the opportunity to manipulate and study the functional relevance of ongoing cortical activity (Thut et al., 2011). Additional limitations when studying how the tACS-frequency interacts with intrinsic frequencies are that, any effect under study occurs within a relative limited frequency band, is thought to have a functional relationship with the applied signal or its harmonics and sub-harmonics, and the function being investigated has little or no relationship to frequencies other than the applied rhythm. One important aspect of the impact that tACS (compared to tDCS) has on the targeted neural population is that depolarisation following polarisation means that any reported effect is not due to any build up in neuronal excitability. Rather, phase alignment, or entrainment, is thought to occur between the phase of the oscillating current and the ongoing neural activity.

1.5 The phase of tACS

Phase alignment, in the context of this study, can be considered a realignment of ongoing intrinsic neural activity, that is rhythmic in nature, to an externally applied alternative rhythmic source (Thut et. al, 2011). This definition describes the changing of a usually intrinsic neuronal rhythm by an external rhythm such that neuronal activity is changed to follow the periodicity of the new rhythmic source. Though this external rhythm can be some form of repetitive sensory input (e.g., auditory, visual, or tactile) it also refers the weak electrical current applied during electrical (or magnetic) brain stimulation techniques such as tACS. The intrinsic neuronal rhythm that is purportedly phase aligned through tACS is considered to be a network or population of neurons that can, and do, periodically fluctuate between states of depolarization and hyperpolarization. This fluctuation is represented in EEG and MEG studies as a continuing reversal in the polarity of scalp potentials at electrode sites. This rhythmic change is frequently observed in posterior locations at alpha frequencies occurring spontaneously and autonomously regardless of the presence of external stimulation. Based on the self-sustained autonomy of the alpha rhythm and its state dependence, its very presence acts to indicate it is likely to serve a causal role in functioning of the nervous system. For tACS to successfully realign the phase of the internal alpha rhythm some theoretical considerations need to be taken in to account (see Pikovsky, Rosenblum, & Kurths, 2001 for a detailed description of the fundamental principles involved in synchronization of two separate oscillatory systems).

The general idea is that the closer the tACS frequency is to the intrinsic frequency the greater the likelihood that phase alignment between the two signals will occur. When there is only a slight difference between the two frequencies only a low level of electrical current is required to phase-lock the two signals. Where a larger discrepancy between the tACS signal and the frequency of the internal oscillatory cycle exists then a higher current intensity is needed to align and phase-lock the two rhythms. A potential caveat for the successful induction of an effect by tACS is that the applied stimulation frequency needs to approximately match the intrinsic frequency of the underlying neuronal network of interest (Ali, Sellers, & Fröhlich, 2013; Schmidt, Iyengar, Foulser, Boyle, & Fröhlich, 2014). Taking this important fundamental principle in to account many researchers choose to adopt a protocol whereby an individual's own intrinsic frequency

during a cognitive process of interest is determined prior to stimulation (e.g., Chander et al., 2016; Gundlach et al., 2016; Ruhnau, et al., 2016). In studies interested in the alpha frequency this is commonly referred to as individualized alpha frequency (IAF). Researchers also need to be wary that when the stimulating current is low and the difference in frequencies high it is possible that some form of partial phase-alignment at a frequency between the two signals will occur (Fröhlich, 2015). It should also be noted that these principles also apply when the tACS frequency is at, or near to, a harmonic or sub-harmonic of the targeted rhythm. Taking all this in to account the evidence strongly suggests that, given sufficiently large enough stimulation, a range of frequencies can be applied to a neural network of interest.

Evidence for the successful phase-locking of neuronal populations to alternating electrical signals comes from computer modelling (Kasten, Duecker, Meiser, & Herrman, 2019), as well as from in vitro and in vivo studies. In vitro research has indicated that increased phase dependent spike timing occurs in mouse cortical slices as a measure of stimulation intensity and frequency (Schmidt et al., 2014). Increased phase alignment at low electrical currents has been found in live, anaesthetized rats (Ozen et al., 2010), anaesthetized ferrets (Ali et al., 2013) and in non-human primates (Johnson et al., 2019). These studies offer direct evidence that relatively low external electrical currents at alternating frequencies can phase-lock the activity of intrinsic neuronal rhythms and the networks they comprise. They also indicate that an important consideration for the successful stimulation and phase-locking of ongoing neural oscillations is the relationship between the intrinsic activity and the stimulation intensity and frequency, in line with theories of weak coupled oscillators (Pikovsky et al., 2001).

Research using human subjects has also provided evidence for the phase-locking of intrinsic neural rhythms to repetitive stimuli. Using EEG and MEG to monitor neural activity while a flickering light is viewed, studies have shown that the closer the frequency of the external light-source is to the speed of the intrinsic rhythm the greater the level of alignment between the two signals (Notbohm, Kurths, & Herrmann, 2016). In contrast to repetitive sensory input, such as the photic driving used in these studies, observing neural phase-locking in the EEG whilst applying tACS is slightly more challenging. The electrical signal produced by tACS introduces a strong electromagnetic artefact in both recording methods such that they need to be further processed in order

to be removed (Neuling, Ruhnau, Weisz, Herrmann, & Demarchi, 2017). Specific to the alternating current used during tACS, is that (assuming successful phase-locking) that both the externally applied signal and the intrinsic re-aligned signal would precisely match each other. There is still much debate on how to best remove tACS induced artefacts, with some claiming that unless they can be perfectly removed the likelihood of mis-interpretation of the applied signal as neural activity is increasingly high (see Noury & Siegel, 2018). In order to bypass these interpretational drawbacks, other studies have instead focused on frequency specific changes occurring outside of the artefactual moment as an indicator of phase-locking. Despite these reservations, studies combining EEG and tACS have indicated evidence for phase-locking through post-analysis procedures and behavioral measures.

10 Hz tACS has been shown to elicit enhanced alpha power at posterior regions of the brain (Helfrich, et al., 2014) and tACs at 40 Hz has been linked with an increase in interhemispheric coherence in the gamma range when using a two-pair montage to stimulate both hemispheres in phase (Helfrich, et al., 2014). Additional evidence for the successful phase-locking of neural activity to tACS come from studies using MEG, which is less likely to be distorted by tACS induced artifacts (Neuling et al., 2015; Witkowski et al., 2016). Using tACS at individual alpha frequencies Ruhnau et. al (2016) demonstrated coherence between the phase of occipital alpha rhythm and the applied tACS rhythm (Ruhnau, et al., 2016). Notably, the phase alignment was only observed in the eyes-open condition, providing further evidence of rhythmic alpha's relationship with resting state functional connectivity. Additional cognitive studies using individual theta frequencies have also demonstrated successful phase-locking between hemispheres and a decline in working memory when compared to sham (Chander et al., 2016).

In addition to EEG and MEG, functional magnetic resonance imaging (fMRI) has indicated that changes in the blood oxygenation level dependent (BOLD) response are related to alpha activity phase-locked to a tACS rhythm (Vosskuhl, Huster, & Herrmann, 2016). Resting state functional connectivity has also shown to be affected by tACS depending on the frequency applied (Cabral-Calderin, Williams, Opitz, Dechent, & Wilke, 2016). 10 Hz tACS revealed an increase in connectivity in the BOLD response, whereas 40 Hz tACS resulted in a decrease in connectivity. Studies incorporating alpha tACS and EEG

have demonstrated that the phase of the signal is critical for whether near threshold stimuli is detected (e.g., Gundlach et al., 2016), this is in line with phase studies that relied purely on EEG recordings. Studies in the visual domain have also shown that 10Hz tACS over the occipital cortex leads both to an increase in parieto-occipital EEG alpha activity as well as a phase dependent modulation in the detection of the target, where participants are more likely to perceive the visual stimuli if it occurs at one point of the tACS cycle compared to another point on the cycle, with approximately half an oscillation separating them (Helfrich et al., 2014).

The role of tACS induced alpha oscillations in perception has not only been studied in vision but also in other sensory modalities. Using a tactile paradigm Gundlach et al., (2016) applied near-threshold stimuli to participants index fingers and asked them to report if they felt a tactile sensation while they received either tACS at the individual mu alpha frequency over primary somatosensory cortex or sham stimulation (Gundlach et al., 2016). They found that, although tACS as a whole did not alter their mean perceptual thresholds, the perception of tactile stimuli was dependent on where in the phase of the tACS cycle they were presented. In line with the finding in the visual modality, this result indicates that tACS also affects somatosensory perception by inducing phase-dependent moments of neural inhibition and excitation. The study also highlights the potential importance of applying an individualized alpha frequency to maximize phase-locking effects.

The classification of phase effects produced by tACS are usually based on electrophysical data collected during the stimulation. However, some studies have measured alpha oscillatory effects on perceptions without the use of EEG or MEG. Using three tACS frequencies (individual alpha, 2 Hz below individual alpha, 2 Hz above individual alpha) Cecere et al., (2015) demonstrated that tACS at different frequencies modulates the size of the temporal window associated with the perception of one or two visual flashes coupled with two auditory beeps separated by 100 ms, suggesting that the length of an alpha oscillation (i.e. its frequency) determines how much perceptual information can be processed during a specific time period in a cross modal capacity (Cecere et al., 2015), although to what extent the tACS signal was phase locked when its frequency was above or below participants IAF cannot be verified. This study runs parallel with the separate study by Samaha and Postle (2015) who used EEG to

demonstrate a similar temporal timeframe in the alpha frequency range using a purely visual task.

To sum up, evidence suggests that tACS can phase-lock intrinsic neural frequencies, and when applied at the alpha rhythm, tACS induced alpha oscillations provide further indication that the phase of these oscillations is causally related to perceptual variations in trial-by-trial performance. Research not purely in visual perception, but also in auditory and somatosensory perception also suggest a mediatory role of the alpha phase. The effects demonstrated in various studies suggest that alpha-tACS may follow the "pulsed inhibition" framework (Mathewson et al., 2009) whereby alpha-oscillation acts as a rhythmic filter that "pulses" between the cyclic inhibitory states of the peak and trough and where populations of neurons oscillate between an excitable or inhibited state (Klimesch et al., 2007; Mathewson et al., 2011). There is growing support for phase-locking of functional neural network activity by tACS in the alpha frequency band from both the field of biology and within neuroscientific research. The latter is necessarily indirect, and due to the use of EEG, is more susceptible to artefactual distortions that, by its similarly electromagnetic nature, can lead to interpretational error. This reason goes some way in explaining why most tACS research has focused on its global effect on behaviour and only a relatively few studies have utilized the timing of the tACS signal alone to quantify or infer the phase specific effects of alpha. However, with the introduction of new technologies, the potential to take greater advantage of the timing of tACS phase may be possible.

2. Improving timing protocols

In a modern lab, presentation of stimuli (visual, auditory, tactile) relies mostly on a computer mediated setup. This setup can introduce delays in actual presentation, and response timing due to resource allocation of the operating system used, that is largely out of the control of the researcher. This often makes it difficult to rely on any timing measurements taken, especially when multiple devices are connected and vying for processing power (see Salmon et. al, 2017 for a recent discussion). One goal of the current research project is to successfully reduce this delay in recorded timing measurements and stimuli presentation inherent in standard experimental procedures

(i.e. the use of PCs) so that a reliable measure of when stimuli is presented can be logged. A possible remedy for these issues has been previously discussed and implemented (see ten Oever, de Graaf, Bonnemayer, Ronner, Sack & Riecke, 2016), however the methods proposed here may offer more flexibility and incorporate more commonly used software and hardware.

Many neuroscience experiments use a PC which interfaces with external hardware through a port connection (parallel, serial, USB). The PC is usually connected to additional peripheral devices that output stimuli (tactile, visual, auditory) and receive responses (keyboard, vocal). Additional proprietary cables may be used for EEG recording equipment (e.g. ActiveTwo system, BioSemi, Amsterdam), which send markers indicating when experiment specific events occur (e.g. onset of stimuli). The timing of these outputs and inputs is usually controlled and logged using software (e.g. E-Prime, MATLAB) running on a Windows or MAC operating system. In addition to EEG acquisition, some experiments also incorporate additional hardware such as the Neuroconn DC Plus stimulator (NeuroConn Ltd., Ilmenau, Germany) used for tDCS or tACS. When sending out tACS frequencies the Neuroconn DC Plus can be programmed to send an analog pulse at a specific phase of the frequency. If the PC inputs and outputs were instead mediated by a device not constrained by the PCs resources, then processing issues that may affect timing measurements would be negated. Though the tACS hardware does not directly interface with a PC, if its analog pulse signal could be precisely timestamped the phase of the tACS signal could then be associated with other ongoing activities.

To this end the current research project implemented the Chronos (Psychology Software Tools), which connected all the hardware used in this project. The Chronos was still connected to and controlled by a PC running E-Prime, however any activity outputted or inputted can be logged by its internal clock with ms accuracy. Though few studies have reported the integration of the Chronos in to their experimental setup it has successfully been implemented to reduce latency in studies using auditory stimuli (Babjack et al., 2015) and additional authors note its potential value in improving timing accuracy (Plant, 2016). Although the Chronos timing mechanism is independent from the PC, inputs and outputs from the various hardware components are designed to interface with PCs, and so in order to successfully implement their use with the Chronos

(and bypass any computer timing issues) their signaling protocols require decompiling and re-routing through the Chronos.

The Chronos can also receive analog inputs such as those produced by the Neuroconn DC Plus stimulator. The Neuroconn DC Plus can be programmed to send an analog pulse at a specific phase of the programmed tACS signal. This can be useful if any connected apparatus can timestamp with a great deal of accuracy the moment that this pulse is sent (something which the Chronos can provide).

This setup, once implemented, can be utilised in numerous research paradigms, allowing an additional level of detail to be extracted regarding phase from studies that incorporate some forms of electrical stimulation. This method bypasses the need to combine EEG recording and tACS in order to analyse phase. The standard way to test for phase differences involves either offline or online EEG/MEG in combination with tACS (Neuling et al., 2017). Offline involves the application of EEG and tACS in separate sessions where any phase specific properties are correlated by a comparison between the two experimental procedures. Notably this relies on a great deal of temporal certainty across the entire experiment. Alternatively, an online combination of EEG and tACS can be used (see Gundlach et al., 2016 for a pertinent example). As previously mentioned, this is not without difficulties, as the electrical signal produced by tACS need to be further processed to remove any artefacts it induces in the EEG recording (Neuling et al., 2017) and there is still much debate on how to best remove tACS induced artefacts (see Noury & Siegel, 2018). With more precise timing equipment, the need for EEG confirmation of phase effects can be removed and studies that incorporate hardware/software compatible with the Chronos interface can be adapted to include it in their setup.

This proposed setup was initially implemented in a registered report (Jones, Yarrow & Silas, 2018) which has already received ethical approval. The experimental procedure of the ongoing study utilises various time sensitive components along with E-Prime and a TactAmp for stimuli presentation and response collection, BioSemi Active Two system for EEG recording and the Neuroconn DC Plus stimulator for the implementation of tACS at IAF, beta (25 Hz) and sham. A pre-experiment EEG recording task determined participants' IAF. Tactile stimuli were sent to either the left and right finger, whilst at the same time markers were sent to the EEG acquisition device logging

the onset of the stimuli. The main experiment consisted of an endogenous and an exogenous tactile attention task where tactile stimuli was presented to either the left or right fingers. tACS at IAF, beta (25Hz) or sham stimulation (where no phase information is available) was delivered by the Neuroconn DC Plus stimulator during both attentional tasks.

In order to extract the phase information from this study the stimuli and markers during the pre-experiment were required to be re-routed and sent simultaneously through the Chronos, thus enabling enhanced control over both signals. The Chronos was also required to timestamp and deliver tactile stimuli during the main experiment. In order to precisely time the stimulation phase of the tACS signal during the tasks and associate the phase with stimuli onset the NeuroConn also needed to be wired to the Chronos. The experiment also required the collection of responses using a voicekey connected to the TactAmp, which the Chronos would also timestamp. The use of the Chronos to timestamp the phase of the tACS signal, the onset of stimuli and RTs, would bypass the operating system allowing for more precise timing measures. Once implemented the phase of stimuli onset can be calculated on a trial by trial basis and performance can be compared between separate phases of the tACS signal.

Importantly, only one hemisphere was stimulated which allows, in theory, contralateral and ipsilateral stimulation effects to be disentangled. The different attentional cueing effects on RTs have been highly documented (see Carrasco, 2014). During endogenous attention, the more informative the cue the faster the RTs for cued compared to uncued targets. Exogenous attention is characterized by a non-informative cue that draws attention reflexively, with IOR (faster RTs for uncued compared to cued targets) observed when the SOA is greater than 300 ms (Klein 2000). Research also shows that the more informative a cue is the greater the level of lateralised alpha power contralateral to the cue at the somatosensory cortex (Haegens et al., 2011). Based on these findings, the endogenous task of the present study utilised a cue that was 75% informative of the target location, the cue in the exogenous task was uninformative (50%) and the SOA for both tasks was 900 ms. The stimulation at IAFs in the current study is expected to interfere with the facilitation effect found in endogenous tasks for cued targets contralateral to simulation by interfering with the lateralization of alpha power. As exogenous attention has been shown to produce little or no alpha

lateralisation and given the SOA of the current study, IOR is predicted, but is not expected to be affected by stimulation during the exogenous task. The current study also employs 2 control measures; sham stimulation and beta stimulation (25 Hz). Both controls are expected to reproduce standard attentional cueing effects as the sham condition provides no stimulation and the beta frequency occurs outside the alpha attentional network of the somatosensory cortex. However, these predictions are based on changes to lateralisation of power and not trial by trial variations in performance due to phase differences at stimuli onset. The goal of the new setup was specifically to create a method whereby the phase of stimuli onset could be measured. tACS to one hemisphere is expected to not just interfere with power lateralisation at the somatosensory cortex, but also to entrain the alpha frequency. With the new setup the phase position of the tACS signal throughout each trial is accurately recorded and associated with stimuli onset enabling RTs for both the exogenous and endogenous task to correlated with their respective phases during both the alpha and beta tACS cycle.

The majority of phasic research has focused largely on the perceptual processes rather than attentional (e.g. Gundlach et al., 2016). Much like the findings in these studies of perception, we expect that the phase of alpha oscillations to be associated with RT performance, with the fastest and slowest RTs separated by half a tACS cycle when binned according to their phase position. As previous research indicates contralateral changes both in neural activity during spatial attention tasks and in perceptual tactile processes (Gundlach et al., 2016), we expected to see differences between phase bins in both tasks for this condition and not ipsilaterally. Studies evaluating the phase dependence effects for uncued stimuli have found either no significant phase modulation (Busch and VanRullen, 2010) or, more recently, only in the theta rhythm band or at high alpha/low beta frequency ranges (Harris, Dux & Mattingley, 2018). Therefore, we expected no effect of phase for uncued targets in the exogenous task at either frequency.

The majority of research indicates the beta rhythms' global role to be involved in movement and is more associated with the motor cortex than the somatosensory areas (Jensen et al., 2002). Some evidence does suggest that low beta (i.e. below 20 Hz) may also be modulated by phase (Baumgarten Schnitzler & Lange, 2015; Klimesch et al., 2007; Mathewson et al., 2009; Mathewson et al., 2011; Mazaheri & Jensen, 2010),

however given the use of 25 Hz in the current study, no effect of phase is expected during beta stimulation.

The centralisation of the standard stimuli outputs and response inputs, with the addition of ms accuracy in the measurement of tACS stimulation all drawn together allowed for a greater level of certainty in the interpretation of results specific to temporal investigations. Both the tACS delivered at IAF and at beta provided phase specific information that could be correlated with stimuli times. Thus, providing an opportunity to test the phase specific properties of both frequency ranges in relation to the cortical areas the electrical stimuli was applied.

2.1 Methodological considerations

If one is interested in investigating phase, then an extremely high temporal acuity is required. Therefore, the first step of this project was to ensure this, by adapting the hardware and software set up of the ongoing registered study so that the timing of the tACS phase signal could be extracted. Although some minor changes to the programming were required, one stipulation was that no fundamental changes could occur to the experimental procedure that would alter the specific paradigms used, what they initially set out to measure, or introduce any potential or unexpected confounds. Essentially any changes that could be made needed to be non-cosmetic, occurring "under the hood" without altering any prerequisites of the original design (Jones et al., 2018). Once this had been achieved, the next stage was recording data from participants, followed by processing of the data and then analyses.

When initially conceived the consensus was that all data could be analysed using a number of three-way repeated measure ANOVAs with 6 equally spaced phase bins (covering the 360° cycle of a waveform) as a factor included in all analyses, with additional factors Frequency (IAF, beta), Task (endogenous, exogenous) and Stimuli to Hemisphere Relationship (cued contralateral stimulation, cued ipsilateral stimulation, uncued contralateral stimulation to cue, uncued ipsilateral stimulation to cue) incorporated to identify various interactions. However, some additional considerations indicated this form of analysis would be insufficient.

The weighting of trials in the endogenous task (75% cued, 25% uncued) meant that, a relatively small number of trials would sit in each of the six phase bins for uncued

trials (M = 10.67; initial viewing of data confirmed that, once binned, some participants had less than half the mean number of trials per bin, with some having no trials) the number of trials would most likely be reduced once cleaning was applied, therefore unattended data in the endogenous task was not investigated.

Although the prospect of revealing a preferred phase bin across the two attentional tasks of the study is intriguing, if any effect of phase is only present in one attentional component there is a great risk that random trial by trial variations, not related to phase, may obscure any potential findings. In addition, as IAF and beta sessions were carried out separately, there is no guarantee that electrode placement for each participant will be anatomically precise across each visit (leading to slight variations in the area of neuronal stimulation between sessions). Furthermore, some participants did not complete both stimulation sessions, and with variations in performance some or all data was required to be excluded (see Behavioral rejection criteria). With additional manipulation of data through binning and further removal to facilitate timing analyses the number of trials per phase bin would be further reduced, creating some bins where participants may be deemed to have too few trials suitable to analyze.

Based on these considerations the decision was made to separate conditions prior to analysis, creating 6 separate analyses per stimulation session (see Table 1); 2 for endogenous attention (cued targets with contralateral stimulation, cued targets with ipsilateral stimulation) and 4 for exogenous attention (cued targets with contralateral stimulation, cued targets with ipsilateral stimulation, uncued targets with stimulation contralateral to the cue, uncued targets with stimulation ipsilateral to the cue). This also meant that rather than excluding a participant completely due to too few trials in one condition, they may still have provided enough trials to be analyzed in other conditions. These choices informed out hypotheses and allowed for more statistical power.

3. Hypotheses
3.1 Alpha phase
RTs are expected to differ across phase for contralateral cued stimulation to target when binned at target onset for both endogenous and exogenous tasks. Maximal differences

are expected between bins adjacent to the slowest (phase-aligned to 0° bin) and the 180° bin. No prediction is made for the effects of target onset phase for uncued targets in the exogenous task.

3.2 Beta phase

RTs are expected to show no difference across phase for cued or uncued stimulation when binned at target onset for both endogenous and exogenous tasks.

These hypotheses (see Table 1) concerning the phase dependence of tACS induced attentional performance are based on the theories of periodic sampling, sensory gating, and timed inhibition, where it is proposed that the efficient processing of tactile information is relevant to the phase of an alpha oscillation.

Table 1. Separate analyses and predicted outcome for each stimulation condition. Each stimulation session had 6 separate analyses; 2 for endogenous attention (cued targets with contralateral stimulation, cued targets with ipsilateral stimulation) and 4 for exogenous attention (cued targets with contralateral stimulation, cued targets with ipsilateral stimulation, uncued targets with stimulation contralateral to the cue, uncued targets with stimulation ipsilateral to the cue). Uncued targets during the endogenous task were not analysed. An effect of phase was only expected during alpha stimulation for cued target in both the endogenous and exogenous task, when RTs are binned to target onset.

|	Alpha (IAF)		Beta	
	Endogenous	Exogenous	Endogenous	Exogenous
Cued targets with contralateral stimulation	Effect of phase	Effect of phase	No prediction	No prediction
Cued targets with ipsilateral stimulation	No prediction	No prediction	No prediction	No prediction
Uncued targets with stimulation contralateral to the cue	Not analysed	No prediction	Not analysed	No prediction
Uncued targets with stimulation ipsilateral to the cue	Not analysed	No prediction	Not analysed	No prediction

4. Chronos integration

4.1 Methods

Hardware setup

The Chronos is essentially a central clock. It can take inputs from a range of different sources such as serial and parallel connections, USB, microphones etc. The Chronos can also output information. In its simplest set up, a researcher can use the Chronos to time stamp the presentation of auditory stimuli presented to headphones, and time stamp a response on a keyboard. They can then be confident the timing information set and recorded in the software (e.g., E-Prime) is accurate without potential added timing variability which often is included in sound card processing and USB ports.

Although the Chronos timing mechanism is independent from the PC, the inputs and outputs from the various hardware components are designed to interface with PCs, and so in order to successfully implement their use with the Chronos (and bypass any computer timing issues) their signaling protocols needed to be decompiled and re-routed through the Chronos. This process involves the removal of cable interfaces and testing wiring in order to detect which wires are used in the signaling protocol. Both the cable that sends EEG Markers and the cable that sends stimuli and receives response information use 25 pinned parallel cables and signals sent and received can use either a single pin or a combination of some or all pins. Once the specific pin configuration for each experimental component is ascertained then the input and output pins can be attached to the Chronos via a parallel breakout board. The methods used to integrate and interface the Chronos are outlined below in sufficient detail to allow replication of the set up in a different lab.

Parallel cable connections.

Using a parallel breakout board (see Figure 4) and copper wire cable is the simplest way to connect to the Chronos. The 8 pins (D0-D7) are used by the parallel cable to send binary signals, where D0=1, D1=2, D3=4, D4=8, D5=16, D6=32 and D07=64. S3-S6 are used to receive signals.
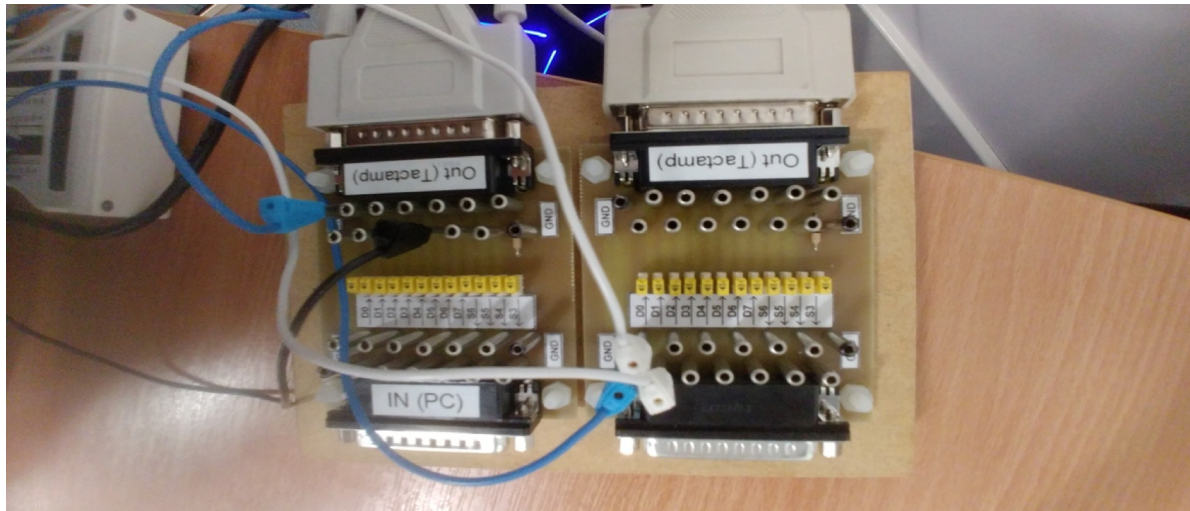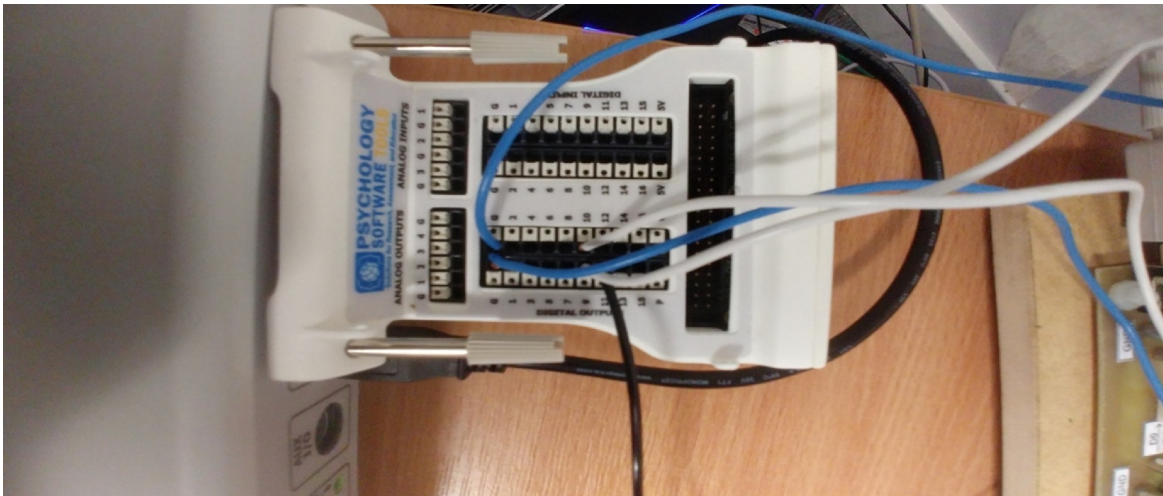

Figure 4. Parallel cable breakout board with Biosemi cable attached (top left) and TactAmp cable attached (top right). The Biosemi cable is attached to pin D6 (black cable) and the TactAmp cable is attached to pins D0 and D1 (white cables). Any signal sent is grounded (blue cables).

The Biosemi Cable and the TactAmp Cable were removed from the PC and attached to the breakout board (see Figure 4). The Biosemi cable uses different pins (or a combination of pins) depending on the required value to be sent as a marker. In Figure 4 the pin at D6 is being used. When set to an "on" state a binary electrical signal will be sent along the copper cable in to pin D6 which will then send a marker value of 32 to the connected EEG recording system until the state is switched off. The TactAmp cable uses pins D0 and D1 to control port 1 and port 2 which are used to output tactile stimuli. When set to an "on" state the binary signal will initiate the desired port's tactile stimuli until an "off" signal is received. Grounding connections are built in to parallel cables, so the ground was also attached for communication to be successful.

Wiring to the digital outputs.

Once the desired wires were connected to the parallel cable pins the other ends were wired to the Chronos. To achieve this, a Chronos expander (Figure 5) was attached to the Chronos and connections were wired directly to it. To attach a bare wire to the Chronos expander, the white numbered block was pushed down, and the wire was inserted downwards and then the block was released. The ground cables were attached to the ground positions.

Figure 5. Wired Digital Outputs of the Chronos expander. Wires from the parallel cable breakout board connected to the Biosemi cable were attached to *Digital Output 11* (black cable). Wires from the parallel cable breakout board connected to the tactamp parallel cable were connected to *Digital Outputs 9* and *10* (white cables). Grounding was connected to *Digital Output* ground (blue cable). *Digital Inputs* are housed above the wired *Digital Outputs*.

Chronos and Neuroconn DC-Stimulator integration.

The Chronos also provides an option to receive analog inputs which, when coupled with devices that transmit analog signals, provides an opportunity to read these signals with a greater level of timing accuracy. One such device is the Neuroconn DC Plus stimulator. When sending out tACS frequencies the Neuroconn DC Plus can be programmed to send an analog pulse at a specific phase of the frequency. This can be useful if any connected apparatus can timestamp with a great deal level of accuracy the moment that this pulse is sent (something which the Chronos can provide).

A BNC cable was attached to the port labelled "Trigger Out" on the Neuroconn DC-Stimulator and the other end of the cable was cut and unsheathed so that the inner

and outer wires could be manipulated (the inner wire is the signal wire and the outer wire is the ground). The signal wire was attached to *Analog Input 2* (See Figure 6), and the ground wire (this was twisted around itself, so it was easier to attach) was attached to the ground (marked as "G" on the expander). Importantly *Analog Input 2* was used as *Analog Input 1* has shorting protection (which we did not require as our setup was safely grounded) that negates the trigger out signal of the DC-Stimulator.

Figure 6. Wired Analog Input 2 of the Chronos expander. The BNC cable (black) was cut and unsheathed. The live cable was attached to Analog Input 2 and the ground wire was attached to the ground.

Software setup

Controlling the Digital Outputs with E-Prime.
Once external devices were connected through their parallel cable interfaces to the Chronos they could then be controlled (activated or deactivated) using inline script (E-Basic) compiled on a PC using E-Prime. The *Digital Outputs* use zero logic so position 1 on the Chronos Expander is referred to as 0 in the script. Position 2 is referred to as 1 and so on.

To turn Digital Output 11 ON the command used was -

Chronos.DigitalOut.Setbit 10

To turn *Digital Output 11* OFF the command used was -

Chronos.DigitalOut.Resetbit 10

In the setup laid out in *Figure 5* these commands turn on and off respectively a digital marker of 32 on the EEG recording system by controlling a signal through Digital Output 11 to pin D6 of the Biosemi cable. Any of the *Digital Outputs* can be used as long as the script used is adjusted accordingly.

Example Script

Chronos.DigitalOut.SetBit 10
Chronos.DigitalOut.SetBit 8
sleep 100
Chronos.DigitalOut.Resetbit 8
Chronos.DigitalOut.ResetBit 10

This script sends a 100 ms marker to pin D6 through the Biosemi cable (see Figure 5) to the attached EEG equipment whilst instantaneously sending 100 ms tactile stimulation to pin D0 and through TactAmp cable to port 1 using the wiring example outlined. The marker and stimuli synchronization created through these steps aid in the precise measurements of events that occur during an experimental paradigm. Once the timings for tactile stimuli can be precisely logged, they then can be married with phase specific timings.

Neuroconn DC-Stimulator and E-prime settings.
DC-Stimulator Plus
When using the "sinus" settings of the stimulator the trigger out signal can be enabled, and the phase settings can be adjusted to when in phase the trigger out signal is sent (set to 0° in the present experiment).

E-Prime

The Chronos was added as a device in E-Prime, then the properties for *Analog Input 2* were adjusted as follows;

Sample Rate – 50000.0000

Scaled Min - 0.0

Scaled Max – 1.0

Onset Threshold – 85%

Offset Threshold – 70%

All other settings were left at default.

These settings read the signal coming from the DC-Stimulator via the *Analog Input 2* connection.

Once the settings were changed the signal was tested during a tACS session by recording the analog signal in E-Prime using the following inline text;

Chronos.Analogin (2).Record

Sleep 1000

Chronos.Analogin (2).Stop

With the stimulation on the Neuroconn set to 10Hz and an E-Prime file containing the above inline code running, a text file that shows time stamps of the analog signal for 1 second was created.

With the apparatus used in the current experiment the text file created through this hardware and software setup showed the signal idling at between 0.60 and 0.70 with an increase to 0.95 every 100 ms (these values may vary in other hardware). With the threshold settings used above, if the signal goes above 0.85 then any input detected is logged as an onset and if the signal goes below 0.70 that is logged as an offset.

The Chronos was set as a response device, and an onset from analog in 2 was registered as the keypress "A" by E-Prime. E-Prime was set to log the keypress "A" and a timestamp of the phase position that the DC-Stimulator is set to send a signal out at was recorded for each trial in the integrated setup.

It should be noted that, as the frequency of the tACS signal is pre-programmed for each session, it would be possible to collect a single tACS time stamp and calculate timings of phase based on this single measure for the duration of a single stimulation session. However, our own recordings indicated that the frequency the brain stimulation device had a slight drift, whereby 0° of phase increased by 1 ms approximately every 1.5 trials during alpha stimulation. With each trial lasting approximately 4 s this change would lead to incorrect phase measurements increasing incrementally as the experiment went on if only an initial time stamp of the tACS phase was used for subsequent analyses. Although this drift is a relatively small magnitude away from the targeted frequency, it is recommended that the phase of stimuli onset is calculated on the trial by trial basis used in this study.

Other than the implementation of the Chronos (which does not affect the overall design) the experimental procedure is the same as described in the ongoing pre-registered report it was employed in (Jones et al., 2018).

A pre-experiment EEG recording task determines participants' individual alpha frequency (IAF). Tactile stimuli were sent to either the left and right finger, whilst at the same time markers were sent to the EEG acquisition device logging the onset of the stimuli. With the new setup, the stimuli and markers were sent simultaneously using the Chronos for both, thus enabling enhanced control over both signals. The main experiment also sent tactile stimuli to either left or right fingers, whilst tACS at IAF, beta (25Hz) or sham stimulation (where no phase information is available) was delivered. As in the pre-experiment the Chronos delivered the stimuli. The NeuroConn was set to send an analog trigger-out signal every 0° phase of the tACS signal to the Chronos, enabling precise timing measurements of the stimulation phase which could then be associated with stimuli onset. The experiment also required the collection of responses using a voicekey, which the Chronos also timestamped – recording RTs that bypass the operating system.

Based on the above setup, timestamps of 0° phase of the tACS signal, the onset of stimuli and RTs were collected for each trial individually and phase of stimuli onset was calculated on a trial by trial basis.

5. tACS and attention

5.1 Methods

Data collection was carried out over 3 separate sessions, set at least 12 hours apart to avoid any possible stimulation carry over effects. The first session consisted of a pre-experiment where participants' IAF was determined, followed by the first stimulation session (alpha/25Hz beta/sham). All participants were randomly assigned a stimulation order and to be stimulated hemisphere (left/right; this allocation was counterbalanced across participants) prior to testing. During stimulation participants undertook an endogenous and exogenous tactile attention task, with a short break in between. Subsequent sessions consisted of the next assigned stimulation to the same hemisphere, with the order that the attentional tasks were carried out reversed.

Participants

The participants were recruited via the university recruitment system (SONA), through local advertisements and via word of mouth. Participants were aged between 18 and 40 years old, had normal (or corrected to normal) vision and were required to be right-handed. Prior to each session participants completed a safety questionnaire adapted from the Transcranial magnetic stimulation (TMS) Adult Safety Screen (TASS) questionnaire (Keel, Smith & Wassermann, 2000). Overall 33 participants took part in at least one session (21 male; 12 female; mean age 23.5 years). All participants received a monetary incentive for their time. The study was approved by the Psychology Department Ethics committee, Middlesex University. All participants were given an information sheet and provided written informed consent prior to taking part.

Materials and apparatus

Two separate PCs were used; one to record EEG data and one for stimulus presentation and recording of behavioural responses. tACS was delivered by a DC-Stimulator Plus (neuroConn©). The presentation of tactile stimuli was carried out by E-Prime v.3 software connected to a TactAmp (Dancer Design, Ltd) via the configured Chronos. The RTs during both tasks were recorded using a voice-key connected to the TactAmp and time-stamped

by the E-Prime connected Chronos. The tACS phase signal and timings of EEG markers were also time-stamped by the E-Prime connected Chronos. The two tactors were set 60 cm apart in front of the participant. Tactile stimuli in the pre-experiment was presented using one tactor contralateral to that participant's to-be-stimulated hemisphere and in the main experiment to both left and right tactors. Speakers played white noise (at a comfortable listening level) to mask any sounds made by the tactors. Black fabric was used to cover participants' hands throughout the experiment, to avoid visual input of the stimulated site (Sambo, Gillmeister, & Forster, 2009). During the pre-experiment, EEG (BioSemi Active Two system) was recorded from 64 active electrodes on the scalp with a sampling rate of 2048Hz. Two vertical electro-oculogram electrodes (VEOG) were placed above and below the right eye. The standard BioSemi reference, Common Mode Sense (CMS) electrode and Driven Right Leg (DRL) electrode were used during recording. During tACS two 70 X 50 mm rubber electrodes were held in place on the scalp using Velcro® strips and/or medical bandaging and conductivity was increased using a Ten20 Conductive paste.

Pre-experiment

Design and procedure

The pre-experiment was adapted from the method used by Gundlach et. al (2016) to establish participants' IAFs. Tactile stimulation involved 100 ms taps to either to the left finger or right index finger, contralateral to which hemisphere tACS would be applied. Prior to the recording, participants were seated comfortably in front of a PC monitor with both their index fingers placed on the two tactors. Once recording started participants were first asked to blink 10 times (prompted by a visual cue on the monitor) so that the recorded activity could be used for ocular correction. Participants then undertook two blocks, with a short break in between, consisting of 150 taps with a mean inter-stimulus interval of 2050 ms and a maximum jitter of 900 ms. A fixation cross was presented on a monitor throughout the blocks.

EEG recording and analysis pipeline

Data was analysed offline via a pre-programmed analysis pipeline (Brain Vision Analyzer 2). Raw data was filtered using 0.1 high- and 40Hz low pass zero phase shift Butterworth

filters, and a 50Hz notch filter. Any bad channels were replaced using topographical interpolation, limited to a maximum of five channels and excluding electrodes C3 and C4 which were required for subsequent analysis. Data was re-referenced to a common average. Ocular correction Independent Component analysis (ICA), based on the blink time interval, was applied to reduce eye-blinks. Data was segmented into 3000 ms long epochs, 1500 ms before and 1500 ms after stimulus onset. A 100 ms pre-stimulus baseline correction was applied. Trials including artefacts, +/-100μV at any electrode were marked as bad and not analysed. Following this a time-frequency analysis was run on the data (excluding bad segments) which had not been baseline corrected. A Complex Morlet wavelet analysis was used (c=5) on the frequency interval between 5-20 Hz, in 150 linear frequency steps (at 0.1 Hz increments). The wavelets analysis was baseline corrected from -600 to 200 ms pre-stimulus interval, avoiding border and smearing effects. The output was spectral amplitude (μV). The data was then averaged across trials and conditions and exported to Matlab where the IAF was determined. An individual participant's IAF was defined as the frequency within the 8-14 Hz range that showed the lowest spectral amplitude in the time window between 200 and 600 ms after stimulus onset at the electrode over the somatosensory cortex (electrodes C3 or C4) contralateral to the hand where stimuli were presented (see Figure 7). This value was used as the stimulation frequency during participants' alpha stimulation tACS session.
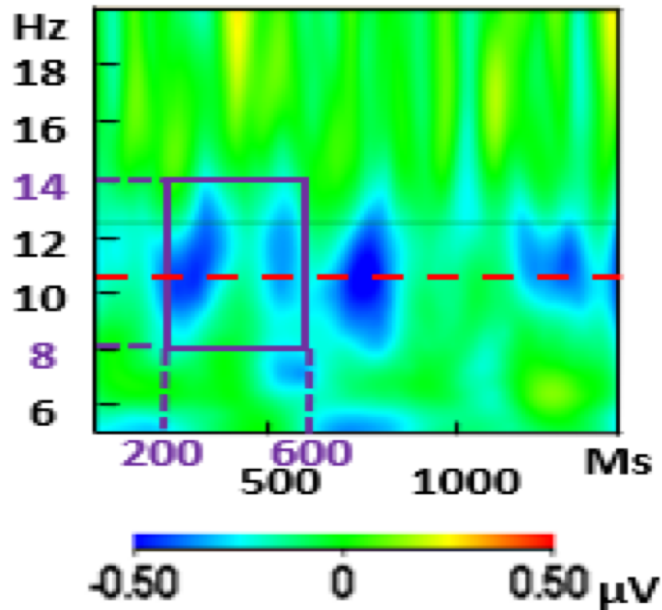
Figure 7. Exemplary EEG output. An individual participant's IAF (red dotted line) was defined as the frequency within the 8-14 Hz range that showed the lowest spectral amplitude in the time window between 200 and 600 ms (purple rectangle) after stimulus onset at the electrode over the somatosensory cortex contralateral to the hand where stimuli were presented.

Main experiment

Design and procedure

tACS stimulation was delivered by DC-Stimulator Plus (ELDITH, Neuroconn, Ilmenau, Germany) via two rubber electrodes (70 × 50 mm). A current of 2 mA, peak to peak, was applied with a maximum current density of 0.5714 A/m2. The impedance was kept below 10 kΩ by applying electrode gel (Ten20, D.O.Weaver, Aurora, CO, USA) between skin and electrode. Participants received stimulation during both endogenous and exogenous tasks of the main experiment (but not during the practice). This is equivalent to two stimulations during each session of less than 30 minutes each, i.e. a maximum of 1 hour per day. This is in line with recent recommendations (Antal et al., 2017). The sham session (not analysed) consisted of stimulation for 30s at 10Hz ramped up to its full current density then ramped down then turned off (Siebner et al., 2004)
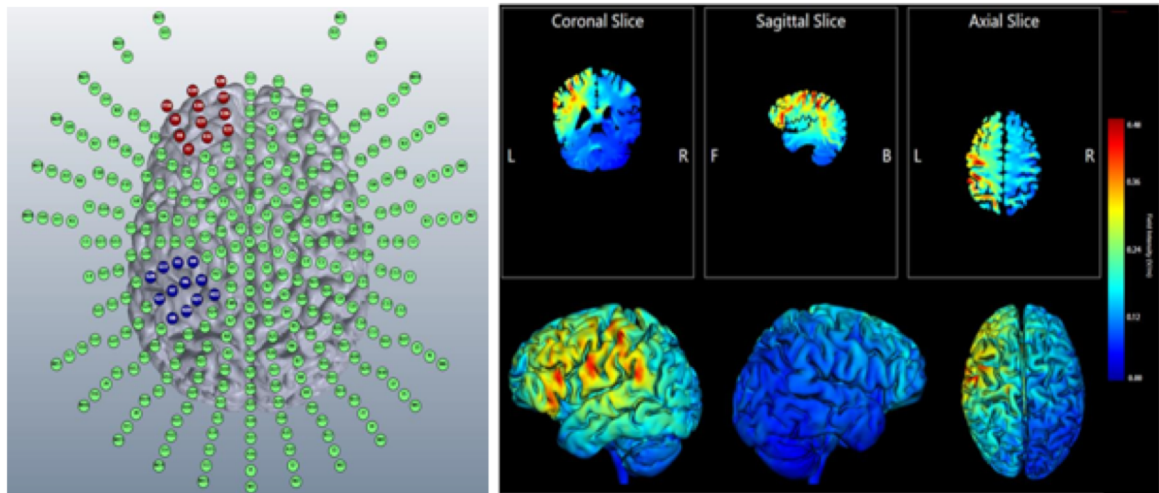
Figure 8: **Left**: Placement of electrodes on the surface of the left hemisphere for use in modelling current flow. 12 ring electrodes were used to approximate the position of the 70 X 50 mm pad electrodes. One pad electrode was modelled at electrode location Cp3 and the other at electrode location Fp1 (Cp4 and Fp2 were used fir the right hemisphere). The frontal electrode was orientated such that the 70 mm side was parallel with midline and the parietal electrode was orientated such that the 50 mm side was parallel with the midline. **Right**: Modelling of stimulation of current field intensity in the brain based on specified electrode locations with 2mA input. 2D slices (top) are centred around the peak voxel of the primary somatosensory cortex (+/ 39, -24, 59). 3D models show the stimulation of current field intensity in the left hemisphere (bottom left), right hemisphere (bottom middle), and the top view of the cortex with the frontal lobe at the top (bottom right).

Field intensity modelling

Simulated theoretical models of current flow patterns in an example 'standard' brain using specialised software (HD-Explore, Soterix Medical) were carried out (see Figure 8). Multiple considerations of electrode placement configurations on the scalp were simulated and field intensity was computed based on the model. The primary aim was to achieve a maximal field intensity at the peak voxel of the primary somatosensory cortex over one hemisphere (+/-39, -24,59) as defined by activation likelihood estimations (Mayka, Corcos, Leurgans & Vaillancourt, 2006). Secondly, the aim was to ensure limited current field intensity over the same coordinates over the opposite hemisphere, in order to ensure stimulation of the primary somatosensory region was uni-hemispheric. Finally, minimal current field intensity over the visual cortex was required, given the role of alpha oscillations in visual processing (+/-11, 81,7; Lacadie, Fulbright, Arora, Constable, & Papademetris, 2008). An approximation of the stimulation delivered via 50 X 70 mm electrodes was achieved by selecting 12 ring electrodes on a 322-electrode montage. The criteria were best met when one pad electrode was modelled at electrode location Cp3 and the other at electrode location Fp1 (for the left hemisphere). The frontal

electrode was orientated such that the 70-mm side was parallel with the midline whereas the parietal electrode was orientated such that the 50-mm side was parallel with the midline. These parameters resulted in a field intensity of 0.229 V/m at the peak-voxel of the primary somatosensory cortex on the left hemisphere, 0.105 V/m at the peak-voxel of the primary somatosensory cortex on the right hemisphere and 0.103 V/m and 0.120 V/m over the left and right primary visual cortices respectively. Although current, and subsequently field strength intensity, is widely distributed throughout the cortex, these models allowed for the confident claim that only one hemisphere of the primary somatosensory cortex was being manipulated and more so than primary visual areas with the chosen electrode montage. Although, models based on standard brain types are likely to differ from individuals' cortical structure – this modelling informs the methodological approach but does not provide precise current flow maps.

Endogenous orienting task

For a diagrammatic representation of the task see Figure 9. Each trial began with a 100 ms tactile cue to both fingers. If the cue was a single 100 ms vibration to the left finger, it indicated to the participant that they should focus their attention to the left. If the cue was a double tap (two 40 ms taps with a 20 ms inter-stimulus interval; ISI), they should expect the target to the right. Participants were explicitly informed that they should use the cues to shift their attention and expect a target at their cued finger, and that this would speed up their RTs. After an SOA of 900 ms a target (100 ms single tap) appeared to either the cued (75%) or uncued (25%) finger and participants responded as quickly as possible by saying "pa" in a microphone. The relationship between the tactile cue (single tap or double tap) and where to expect the target (left finger or right finger) was counterbalanced across participants. Following their response, a random was an inter-trial interval (ITI) of 1500 to 3000 ms before the next trial. If no response was made within 2000 ms of the target presentation, then the trial was marked as a miss and the ITI commenced. A fixation cross was presented on a monitor throughout each task. Before each task the participants carried out two practice blocks of 19 trials without stimulation.
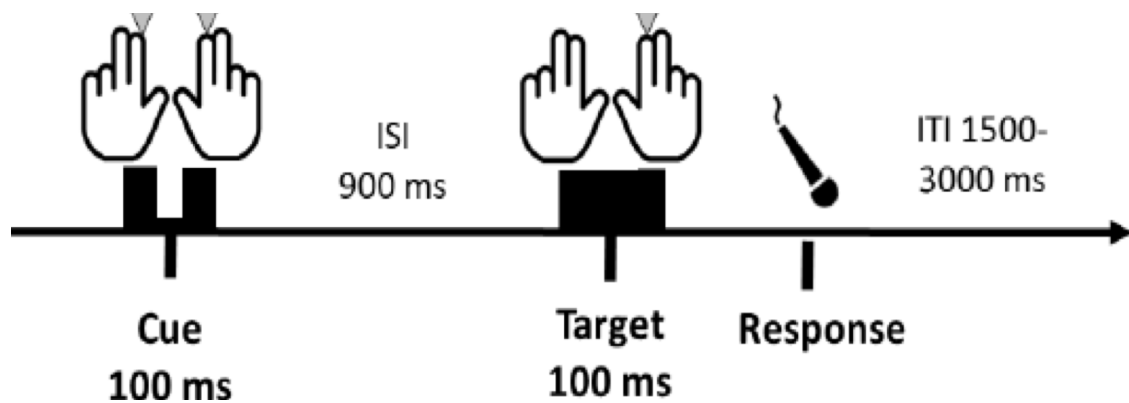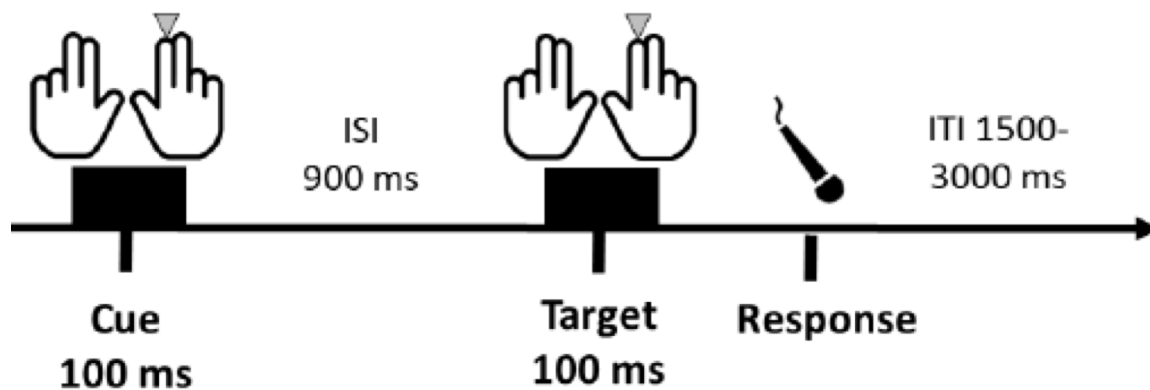
Figure 9. Schematic view of trial events during the endogenous task. Participants received stimulation to one hemisphere throughout the task. Each trial began with a 100 ms tactile cue to both fingers. If this was a single 100 ms tap, it indicated to the participant that they should focus their attention and expect that the next target (a single vibration to one finger) would be to the left finger. A double tap (two 40 ms taps with a 20 ms inter-stimulus interval) indicated that the participant should focus attention and expect that the next target was likely to occur to the right finger. The relationship between the tactile cue (single tap or double tap) and where to expect the target (left finger or right finger) was counterbalanced between participants. After an SOA of 900 ms a target (100 ms single tap) appeared to either the cued or uncued finger and participants responded as quickly as possible by saying "pa" in to a microphone. Following this was an ITI ranging from 1500 to 3000 ms before the next trial. Each block contained; 24 trials cued contralaterally to the stimulated hemisphere, 24 trials cued ipsilaterally to the stimulated hemisphere, 8 trials uncued with the cue contralateral to the stimulated hemisphere and 8 trials uncued with the cue ipsilateral to the stimulated hemisphere.

Practice blocks were composed of 12 cued, 4 uncued, 1 fast filler and 2 catch trials. To ensure reliability between each session, the practice was also carried out in sessions 2 and 3. Practice blocks ensured participants understood the task and were not included in subsequent analysis. In cued trials the cue and target were presented to the same finger, and in uncued cue and target were presented to different fingers. Catch trials consisted of a cue with no target, where the participant was told not to respond. Fast filler trials consisted of a cue and target with a faster SOA (400 ms). Fast filler and catch trials were not analysed, they were only present to ensure that the participant did not anticipate the target or respond automatically (see Jones & Forster, 2014 for a similar design and procedure). The endogenous task was composed of 4 blocks of 76 trials with each block containing 4 fast filler and 8 catch trials. Stimulation to only 1 hemisphere created 4 separate stimulation conditions. Each block contained 24 trials cued contralaterally to the stimulated hemisphere, 24 trials cued ipsilaterally to the stimulated hemisphere, 8 trials uncued with the cue contralateral to the stimulated hemisphere and 8 trials uncued with the cue ipsilateral to the stimulated hemisphere (uncued trials were also not analysed due to insufficient number of trials for phase binning). At the end of each block overall RTs and

errors were displayed on the monitor and participants had a short break before the next block.

Exogenous orienting task

For a diagrammatic representation of the task see Figure 10. The exogenous orienting task followed the same procedure as endogenous task with the following exceptions. For every trial, the cue was a 100 ms single tap to the right or left finger which participants were told did not predict the location of the next target. The practice block was composed of 1 fast filler, 2 catch, 8 cued and 8 uncued trials. The real task was composed of 4 blocks of 76 trials with each block containing 4 fast filler and 8 catch trials. As in the endogenous task stimulation to only 1 hemisphere created 4 separate stimulation conditions. Each block contained 16 trials cued contralaterally to the stimulated hemisphere, 16 trials cued ipsilaterally to the stimulated hemisphere, 16 trails uncued with the cue contralateral to the stimulated hemisphere and 16 trials uncued with the cue ipsilateral to the stimulated hemisphere. Both cued and uncued trials were included in analyses.

Figure 10. Schematic view of trial events during the exogenous task. Participants received stimulation to one hemisphere throughout the task. Each trial began with a single 100 ms tap to the left or right finger, which participants were told did not predict the location of the target. After an SOA of 900 ms a single 100 ms tap (the target) occurred at either the cued or uncued finger and participants responded as quickly as possible by saying "pa" in to a microphone. Following this was an inter-trial interval ranging from 1500 to 3000 ms before the next trial. Each block contained; 16 trials cued contralaterally to the stimulated hemisphere, 16 trials cued ipsilaterally to the stimulated hemisphere, 16 trails uncued with the cue contralateral to the stimulated hemisphere and 16 trials uncued with the cue ipsilateral to the stimulated hemisphere.

6. Data analysis

This experiment aimed to adapt an ongoing study that used tACS and improve timing mechanisms so that the phase of the stimulation signal could be determined during stimuli onset. Reaction times (RTs) in an endogenous and exogenous tactile attention task at 2 different frequencies could then be associated with their respective phase bins and any trial by trial influence of tACS induced phase on performance could be investigated.

2 participants only took part in a sham session and their data was not required for phase analysis. Of the real stimulation sessions 25 participants took part in both alpha and beta stimulation sessions (3 were later found to be left handed and were removed from all datasets) and 1 was removed due to excessive movement during the tasks. 2 participants took part in alpha only, and 4 took part in beta only. Examination of data output files indicated that 1 participant had missing alpha and beta time-stamps and was removed from all analyses and 2 participants had missing beta time-stamps and were removed from all beta analyses.

EEG Rejection criteria

Participants were excluded from the experiment if their frequency could not be defined following the pre-experiment (see *EEG recording and analysis pipeline*). In addition, exclusion also occurred if the artefactual contamination (ocular muscular activity or other) was present in more than 60% of the trials.

Behavioural rejection criteria:

For each stimulation condition, participants' trials with RTs below 100 ms (including no response) and above 2 standard deviation from the individual average of each participant (average calculated after removal of responses below 100 ms and excluding fast filler and catch trials) were eliminated. If this cleaning resulted in a decrease of more than 15% in the total number of trials per stimulation condition (15 trials in endogenous conditions and 10 trials in exogenous conditions), then the participants data were excluded from the analysis of that condition but kept for other conditions. If participants accidentally responded to more than 50% of the catch trials in

a task their data were excluded from that task. As data was excluded by condition only, the participants and their numbers varied from analysis to analysis.

Post-processing of timing measurements

Each trial contained an individual time-stamp for target onset, RT, and 0° phase position of the tACS signal. These data were used to place a participant's response times in to 1 of 6 equally spaced phase bins (0°, 60°, 120°, 180°, 240°, 300°) according to where in phase the target onset was during each trial.

Though the Chronos has ms accuracy, due to the speed of a tACS oscillation, 1ms can mean the difference between a stimulus onset occurring in a particular phase bin or the phase bin adjacent to it. In addition, stimuli onset and RTs were also time-stamped, creating three measures with possible variability issues. To account for this potential variation additional cleaning of binned data was applied.

The fastest tACS frequency used in this study was 25 Hz, which means that a full oscillation occurs every 40 ms. As data was placed in to 6 bins of equal length, each bin lasted 6.67ms. With a 1 ms delay, a slight proportion of time-stamps may indicate onsets belonging to incorrect bins. To combat this, data for each participants' 60° bin was further divided in to 6 bins (10° apart) and the last 10° of the 6 bins were removed. The remaining 5 bins were averaged together to create 6 bins, 50° in length, essentially creating a 10° buffer (lasting 1.11 ms) between phase bins that removed some or all overlap potentially occurring in our timing measurements. The same process was also applied to alpha frequencies creating a slightly greater buffer (due to its slower frequency range) that varied depending upon each IAF. If any bin was unpopulated following binning and removal of the last 10° of each bin in a condition, then participants data was removed from that condition only.

6.1 Alpha

No participant was excluded due to an undefined IAF. The average response to catch trials was less than 2% in all conditions with the largest response rate being 1.3%. No participant was removed due to unpopulated phase bins following binning and removal of the last 10° of each bin.

Endogenous tactile attention

Participant information for each analysis of the endogenous task during alpha stimulation is displayed in Table 2.

Table 2. Participant numbers and mean age (in years) for each analysis of the endogenous task during alpha stimulation.

	Participants	Mean age (SD)
Cued targets with contralateral stimulation	21 (13 Male; 8 female)	24.1 (4.5)
Cued targets with ipsilateral stimulation	21 (13 Male; 8 female)	24 (4.6)

Cued targets with contralateral stimulation

1 participants data was removed from the analysis with more than 15% of trials excluded prior to binning. The mean IAF for remaining participants was 10.9 Hz (SD = 2.3). The mean number of trials per bin was 12.6 (SD = 3.4), with the smallest bin containing 6 trials.

Cued targets with ipsilateral stimulation

1 participants data was removed from the analysis with more than 15% of trials excluded prior to binning. The mean IAF for remaining participants was 10.9 Hz (SD = 2.3). The mean number of trials per bin was 12.7 (SD = 3.2), with the smallest bin containing 6 trials.

Exogenous tactile attention

Participant information for each analysis of the exogenous task during alpha stimulation is displayed in Table 3.

Table 3. Participant numbers and mean age (in years) for each analysis of the exogenous task during alpha stimulation.

	Participants	Mean age (SD)
Cued targets with contralateral stimulation	21 (13 Male; 8 female)	24.1 (4.5)
Cued targets with ipsilateral stimulation	21 (13 Male; 8 female)	24.1 (4.5)
Uncued targets with stimulation contralateral to the cue	21 (13 Male; 8 female)	24.1 (4.5)
Uncued targets with stimulation ipsilateral to the cue	21 (13 Male; 8 female)	24.1 (4.5)

Targets cued contralaterally to the stimulated hemisphere.

1 participants data was removed from the analysis with more than 15% of trials excluded prior to binning. The mean IAF for remaining participants was 10.9 Hz (SD = 2.3). The mean number of trials per bin was 8.5 (SD = 2.7), with the smallest bin containing 2 trials.

Targets cued ipsilaterally to the stimulated hemisphere.

1 participants data was removed from the analysis with more than 15% of trials excluded prior to binning. The mean IAF for remaining participants was 10.9 Hz (SD = 2.3). The mean number of trials per bin was 8.5 (SD = 3), with the smallest bin containing 2 trials.

Uncued targets with stimulation contralateral to the cue.

1 participants data was removed from the analysis with more than 15% of trials excluded prior to binning. The mean IAF for remaining participants was 10.9 Hz (SD = 2.3). The mean number of trials per bin was 8.4 (SD = 2.9), with the smallest bin containing 1 trial.

Uncued targets with stimulation ipsilateral to the cue.

1 participants data was removed from the analysis with more than 15% of trials excluded prior to binning. The mean IAF for remaining participants was 10.9 Hz (SD = 2.3). The mean number of trials per bin was 8.4 (SD = 2.6), with the smallest bin containing 3 trials.

6.2 Beta

2 participants were removed from all beta analyses due to more than 15% of trials being excluded in each condition. The average response rate to catch trials in all conditions was less than 1%. No participant was removed due to unpopulated phase bins following binning and removal of the last 10° of each bin.

Endogenous tactile attention

Participant information for each analysis of the endogenous task during beta stimulation is displayed in Table 4.

Table 4. Participant numbers and mean age (in years) for each analysis of the endogenous task during beta stimulation.

	Participants	Mean age (SD)
Cued targets with contralateral stimulation	18 (12 male; 6 female)	23.7 (3.8)
Cued targets with ipsilateral stimulation	18 (12 male; 6 female)	23.5 (3.2)

Cued targets with contralateral stimulation

2 participants data were removed from the analysis with more than 15% of trials excluded prior to binning. The mean number of trials per bin was 13 (SD = 3.4), with the smallest bin containing 6 trials.

Cued targets with ipsilateral stimulation

2 participants data were removed from the analysis with more than 15% of trials excluded prior to binning. The mean number of trials per bin was 12.9 (SD = 3.4), with the smallest bin containing 5 trials.

Exogenous tactile attention

Participant information for each analysis of the exogenous task during beta stimulation is displayed in Table 5.

Table 5. Participant numbers and mean age (in years) for each analysis of the exogenous task during beta stimulation.

	Participants	Mean age (SD)
Cued targets with contralateral stimulation	16 (11 Male; 5 female)	24.1 (3.9)
Cued targets with ipsilateral stimulation	16 (11 Male; 5 female)	24.1 (3.9)
Uncued targets with stimulation contralateral to the cue	18 (12 Male; 6 female)	23.8 (3.8)
Uncued targets with stimulation ipsilateral to the cue	18(16 Male; 6 female)	23.8 (3.8)

Targets cued contralaterally to the stimulated hemisphere.

4 participants data were removed from the analysis with more than 15% of trials excluded prior to binning. The mean number of trials per bin was 8.7 (SD = 3), with the smallest bin containing 3 trials.

Targets cued ipsilaterally to the stimulated hemisphere.

4 participants data were removed from the analysis with more than 15% of trials excluded prior to binning. The mean number of trials per bin was 8.6 (SD = 2.6), with the smallest bin containing 3 trials.

Uncued targets with stimulation contralateral to the cue.

2 participants data were removed from the analysis with more than 15% of trials excluded prior to binning. The mean number of trials per bin was 8.8 (SD = 2.9), with the smallest bin containing 3 trials.

Uncued targets with stimulation ipsilateral to the cue.

2 participants data were removed from the analysis with more than 15% of trials excluded prior to binning. The mean number of trials per bin was 8.7 (SD = 2.6), with the smallest bin containing 3 trials.

6.3 Phase alignment

A common procedure in studies of phase is the need for phase alignment of each participants data (Mathewson et al., 2009; Busch et al., 2009; Neuling, Rach, Wagner, Wolters & Herrmann, 2012). This is necessary due to interindividual anatomical differences as well as differences in individualized tACS frequencies; the phase at which the participants show the fastest and slowest RTs will vary between them. Therefore, once data binning had been carried out all participants data was individually realigned so that the slowest bin was placed in the 0° bin. As this phase alignment leads to slowest RTs being maximal at the 0° bin this bin was not included in analyses (see Zoefel, Davis & Riecke, 2019 for a detailed account on the possible phase-alignment procedures that can be considered, and subsequent analyses required).

7. Results

Data was analysed using SPSS. 6 analyses for each frequency were carried out; 2 for endogenous attention (cued targets with contralateral stimulation, cued targets with ipsilateral stimulation) and 4 for exogenous attention (cued targets with contralateral stimulation, cued targets with ipsilateral stimulation, uncued targets with stimulation contralateral to the cue, uncued targets with stimulation ipsilateral to the cue). As we expected differences between phase bins, each analysis consisted of each participants' mean RT for phase bins (60°, 120°, 180°, 240°, 300°; 0° bin excluded from analysis) included in a set of 10 pairwise t-tests (Bonferroni corrected p set to .005). Table 6 summarizes the separate analyses and results.

Table 6. Summary of results. An effect of phase was found for targets cued ipsilaterally to the alpha stimulated hemisphere during endogenous tactile attention. An effect of phase was found for targets cued contralaterally to the beta stimulated hemisphere during exogenous tactile attention. All other analyses indicated no significant effect of phase. Uncued targets during the endogenous task were not analysed.

	Alpha (IAF)		Beta	
	Endogenous	Exogenous	Endogenous	Exogenous
Cued targets with contralateral stimulation	No effect of phase	No effect of phase	No effect of phase	Effect of phase
Cued targets with ipsilateral stimulation	Effect of phase	No effect of phase	No effect of phase	No effect of phase
Uncued targets with stimulation contralateral to the cue	Not analysed	No effect of phase	Not analysed	No effect of phase
Uncued targets with stimulation ipsilateral to the cue	Not analysed	No effect of phase	Not analysed	No effect of phase

7.1 Alpha

Endogenous tactile attention

Cued targets with contralateral stimulation

Paired sample t-tests showed no significant difference between all phase bins (all *ps* > .44), suggesting random trial by trial and phase by phase variations in RTs when targets were cued contralaterally to stimulation.
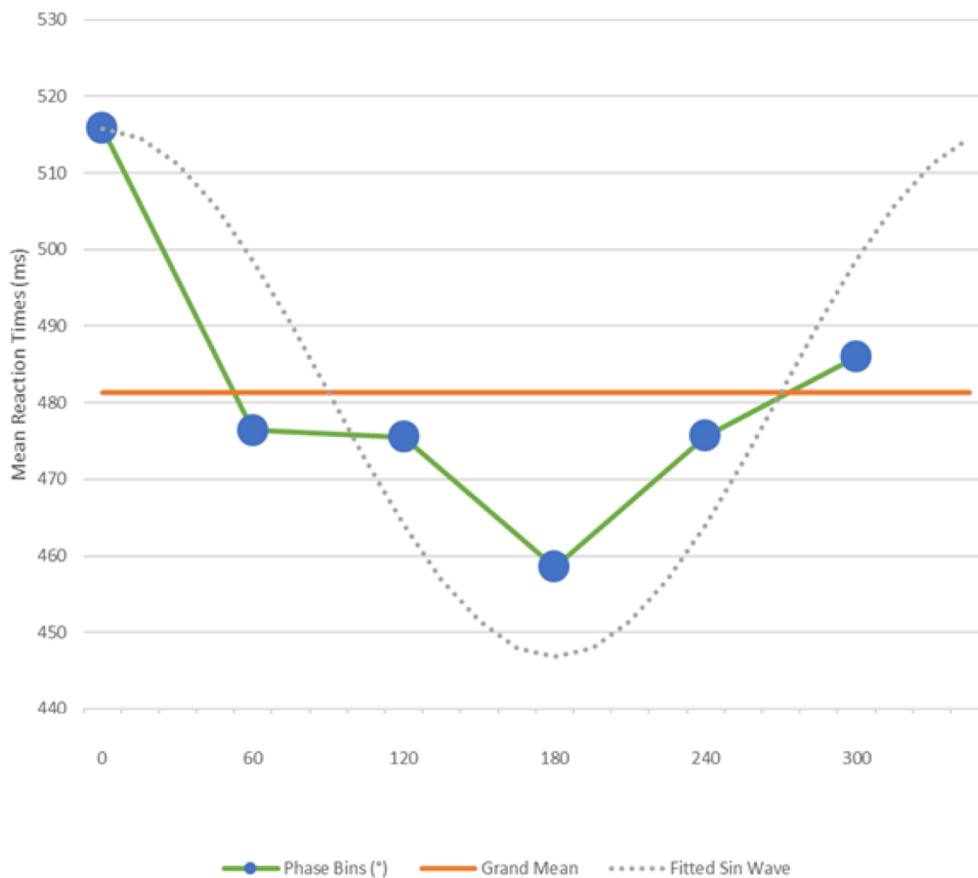
Cued targets with ipsilateral stimulation

Paired sample t-tests indicated a significant difference between the 60° bin (M = 476.37, SD = 158.11; adjacent to the aligned slowest bin) and the 180° bin (M = 458.53, SD = 149.69); $t(20)$ = 3.288, p = .004, Cohen's d = 0.72 (see Table 7). Had the comparisons only included combinations with the 180° bin (the fastest phase bin; situated half an oscillation away from the slowest phase bin) then both the 240° bin (M = 475.61, SD = 157.93) and the 300° bin (M = 485.99, SD = 157.95; adjacent to the aligned slowest bin) would also have been significantly different to the 180° bin; $t(20)$ = -2.928, p = .008, d = -0.64 and $t(20)$ = -2.913, p = .009, d = -0.64 respectively.

Table 7. Paired sample T-Tests for phase bins in the endogenous task with alpha stimulation contralateral to cued targets. A significant difference was found between the 60° bin (M = 476.37, SD = 158.11; adjacent to the aligned slowest bin) and the 180° bin (M = 458.53, SD = 149.69); t(20) = 3.288, p = .004, d = 0.72. Bonferroni corrected p was set to .005.

			t	df	p	Cohen's d
60° Phase Bin	-	120° Phase Bin	0.092	20	0.928	0.020
60° Phase Bin	-	180° Phase Bin	3.288	20	0.004	0.718
60° Phase Bin	-	240° Phase Bin	0.112	20	0.912	0.024
60° Phase Bin	-	300° Phase Bin	-1.258	20	0.223	-0.275
120° Phase Bin	-	80° Phase Bin	1.759	20	0.094	0.384
120° Phase Bin	-	240° Phase Bin	-0.011	20	0.992	-0.002
120° Phase Bin	-	300° Phase Bin	-1.201	20	0.244	-0.262
180° Phase Bin	-	240° Phase Bin	-2.928	20	0.008	-0.639
180° Phase Bin	-	300° Phase Bin	-2.913	20	0.009	-0.636
240° Phase Bin	-	300° Phase Bin	-1.276	20	0.216	-0.279

The resulting distribution of RTs averaged across subjects indicated the slowest and fastest RTs were separated by 180° (i.e. in opposite phase bins of the tACS waveform) and that phase binned RTs approximately followed the pattern of the waveform signal (see Figure 11).

Figure 11. Endogenous task with alpha stimulation ipsilateral to cued targets. Reaction times binned by phase position at target onset. Blue dots represent the mean RTs presented within six different phase bins of the alpha tACS. Dashed line represents an exemplary tACS curve. Orange line represents the grand mean. The distribution of RTs averaged across subjects indicates the slowest (0° bin; not included in analyses) and fastest (180° bin) RTs are separated by 180° (i.e. in opposite phase bins of the tACS waveform) and that phase binned RTs approximately follow the pattern of the waveform signal.

Exogenous tactile attention

Bonferroni corrected paired sample t-tests showed no significant difference between any phase bins in any of the cued (cued targets with contralateral stimulation, cued targets with ipsilateral stimulation; all $ps > .077$), or uncued (uncued targets with stimulation contralateral to the cue, uncued targets with stimulation ipsilateral to the cue; all $ps > .12$) exogenous tactile attention conditions, suggesting random trial by trial and phase by phase variations in RTs for exogenous tactile attention regardless of how attention was oriented during IAF stimulation.

7.2 Beta

Endogenous tactile attention

Bonferroni corrected paired sample t-tests showed no significant difference between any phase bins in both cued endogenous tactile attention conditions (cued targets with contralateral stimulation, cued targets with ipsilateral stimulation; all *ps* > .079) suggesting random trial by trial and phase by phase variations in RTs for cued endogenous tactile attention during beta stimulation.

Exogenous tactile attention
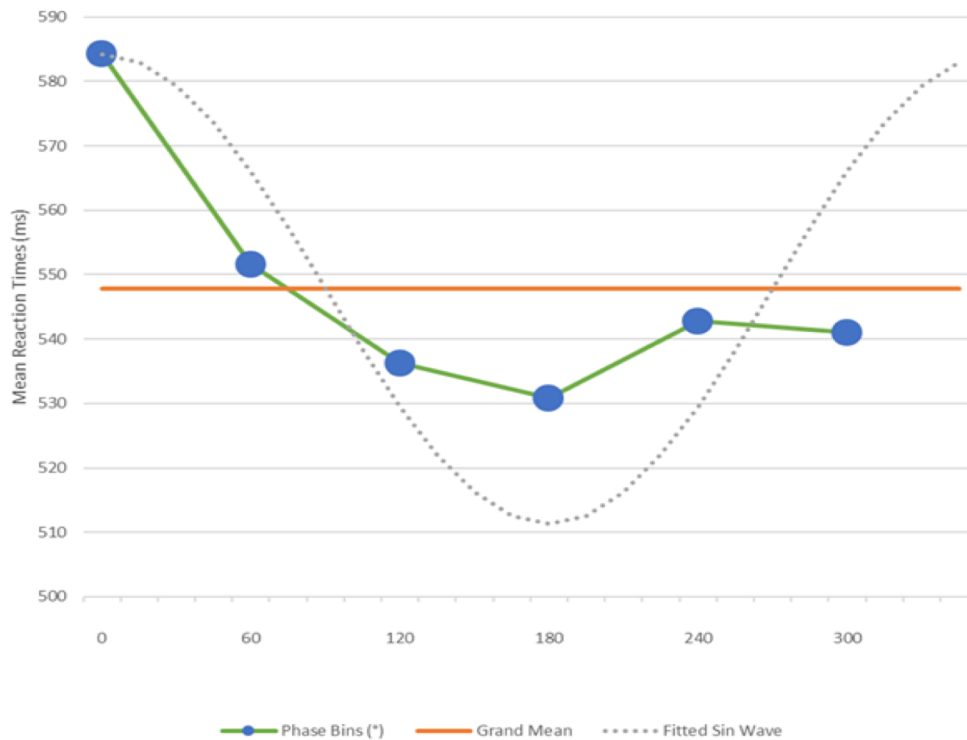
Cued targets with contralateral stimulation

Paired sample t-tests (Bonferroni corrected p set to .005) indicated a significant difference between the 60° bin (M = 551.57, SD = 153.54; adjacent to the aligned slowest bin) and the 180° bin (M= 530.83, SD = 158.72); $t(15) = 3.401$, $p = .004$, $d = 0.85$ (see Table 8).

Table 8. Paired sample T-Tests for phase bins in the endogenous task with beta stimulation contralateral to cued targets. A significant difference was found between the 60° bin (M = 551.57, SD = 153.54; adjacent to the aligned slowest bin) and the 180° bin (M= 530.83, SD = 158.72); t(15) = 3.401, p = .004, d = 0.85. (Bonferroni corrected p was set to .005).

			t	df	p	Cohen's d
60° Phase Bin	-	120° Phase Bin	1.503	15	0.154	0.376
60° Phase Bin	-	180° Phase Bin	3.401	15	0.004	0.850
60° Phase Bin	-	240° Phase Bin	0.853	15	0.407	0.213
60° Phase Bin	-	300° Phase Bin	1.262	15	0.226	0.316
120° Phase Bin	-	180° Phase Bin	0.571	15	0.576	0.143
120° Phase Bin	-	240° Phase Bin	-0.611	15	0.550	-0.153
120° Phase Bin	-	300° Phase Bin	-0.632	15	0.537	-0.158
180° Phase Bin	-	240° Phase Bin	-1.203	15	0.248	-0.301
180° Phase Bin	-	300° Phase Bin	-1.177	15	0.257	-0.294
240° Phase Bin	-	300° Phase Bin	0.245	15	0.810	0.061

The resulting distribution of RTs averaged across subjects indicated the slowest and fastest RTs were separated by 180° (i.e. in opposite phase bins of the tACS waveform; see Figure 12). With the exception of the 300° bin the distribution approximated the waveform of the tACS signal.

Figure 12. Exogenous task with beta stimulation contralateral to cued targets. Reaction times binned by phase position at target onset. Blue dots represent the mean RTs presented within six different phase bins of the alpha tACS. Dashed line represents an exemplary tACS curve. Orange line represents the grand mean. The distribution of RTs averaged across subjects indicates the slowest (0° bin; not included in analyses) and fastest (180° bin) RTs are separated by 180° (i.e. in opposite phase bins of the tACS waveform) and that (with the exception of the 300° bin) phase binned RTs approximately follow the pattern of the waveform signal.

Cued targets with ipsilateral stimulation.

Bonferroni corrected pairwise comparison showed no significant difference between any phase bins (all *ps* > .122), suggesting random trial by trial and phase by phase variations in RTs when targets were cued ipsilaterally to stimulation.

Uncued targets.

Bonferroni corrected pairwise comparison showed no significant difference between any phase bins for both uncued conditions (uncued targets with stimulation contralateral to the cue, uncued targets with stimulation ipsilateral to the cue; all *ps* > .025), suggesting random trial by trial and phase by phase variations in RTs when the cue did not predict the target.

7.3 Peripheral sensations

All participants reported experiencing visual phosphenes, and a general low level of comfortability during the onset of tACS. No participant felt the need to withdraw from a session due to a prolonged discomfort, with all stating that any sensations they felt had subsided quite quickly, although most still reported a slight (but bearable) itching or throbbing throughout each session. However, it should be noted that absences from additional sessions occurred often. This may be attributed to an aversion to additional stimulation, or just to the general requirements of a session (e.g. application of gel, washing and drying of hair, length of session).

8. Discussion

This experiment aimed to adapt an ongoing study that used tACS and improve timing mechanisms so that the phase of the stimulation signal could be analysed. Reaction times (RTs) in an endogenous and exogenous tactile attention task could then be associated with their respective phase bins and any trial by trial influence of phase on performance could be investigated.

Based on comparable research in visual and tactile perceptual studies, that used EEG either alone or in conjunction with tACS (e.g. Busch et al., 2009; Gundlach et el., 2016), an effect of phase was expected to occur during somatosensory alpha stimulation. This was due to the theoretical ability of tACS to entrain intrinsic alpha activity in the somatosensory cortex, thus producing a similar effect to that found in perceptual studies, and thereby providing further evidence for the theories of periodic sampling (Busch & VanRullen, 2010; Schroeder & Lakatos, 2009), sensory gating (Jensen & Mazaheri, 2010), and timed inhibition (Klimesch et al., 2007). These theories propose that the efficient processing of tactile information is relevant to the phase of alpha oscillations. Specifically, we hypothesized that, as stimulation was applied to a single hemisphere, that only when cue and target occur contralaterally to the stimulated hemisphere would an effect of phase be seen in the alpha condition for both endogenous and exogenous attentional tasks. This is based on research that suggests that tactile spatial attention is lateralised in a similar fashion to visual spatial attention

with changes occurring contralaterally to attended regions during endogenous attention and (although to a lesser extent) exogenous attention (Haegens et al. 2011). Our deductions did not preclude Ipsilateral stimulation to cued targets from also being associated with the phase, but rather we would not be able to determine the presence of this relationship based on the measures used. As the tACS setup targeted only one hemisphere the phase of stimuli onset ipsilaterally would not be controlled or measured and any effect of phase would be undetectable. We also expected no effect for uncued targets given the frequency range employed. Research has indicated there may be some evidence for phase dependence towards unattended targets, however this is from only one study were the effects were predominantly in frequency ranges outside the alpha band used in the present study (Harris et al., 2018).

Beta (25 Hz) stimulation was employed primarily as a control measure to determine any differential effect of tACS for the ongoing study (Jones et al., 2018), as some research has shown that tACS at both alpha and beta can increase RTs in motor tasks (Pollock, Boysen & Krause, 2015). Whereas other research has indicated that tACS at alpha leads to faster RTs and tACS at beta leads to RTs increasing (Cappon, D'Ostilio, Garrauz, Rothwell & Bisiacchi, 2016). The majority of research indicates the beta rhythms' global role to be in movement and is more associated with the motor cortex than the somatosensory areas stimulated during this study. Some evidence does suggest that low beta (i.e. below 20 Hz) may also phasically modulate perception, however given the use of 25 Hz in the current study, no effect of phase was expected during beta stimulation. Thus, we hypothesized that RTs were not expected to show a difference across phase for cued or uncued stimulation when binned at target onset for both endogenous and exogenous tasks.

8.1 Phase effects during alpha stimulation

Contrary to our hypotheses, the only significant effect of alpha phase was found for targets cued contralaterally to the non-stimulated hemisphere in the endogenous paradigm.

The effect found in alpha, though not what was predicted, does to some extent confirm previous findings of alpha phase dependence during endogenous visual performance (Mathewson et al., 2011; VanRullen et al., 2011) and tactile perceptual performance

(Gundlach et. Al., 2016). Though not consistent with a great body of work that demonstrates the lateralized effects of alpha, it does however fall in line with the expectations of the theories of periodic sampling, sensory gating, and timed inhibition. Alpha stimulation ipsilateral to cued targets during an endogenous tactile attention task was expected to show no effect of phase, as we assumed that the phase-locking of the uni-hemispheric design would be driven by the causal influence of lateralised alpha found in tactile attention studies (Romei et al., 2010). That is, we expected tACS to replicate the functional mechanisms of the brain leading to phase dependence due to contralateral stimulation of cued targets. The presence of a significant phase effect for targets cued contralaterally to the non-stimulated hemisphere suggests that both hemispheres where phase-locked to the tACS signal, not necessarily in direct alignment with each other, but rather their neural rhythms were entrained by the stimulation. It is possible that phase dependence was present for both stimulated and non-stimulated hemispheres, but due to the small sample size and an inadequate number of trials the analysis could not detect it. Therefor it may be reasonable to assume that the effect found for targets cued to the non-stimulated contralateral hemisphere in the endogenous task would, given a greater sample size, also be present during contralateral stimulation to cued targets and that both hemispheres were in fact phase-locked to the tACS signal.

The seemingly contradicting results showing phase effects in both alpha and beta in attentional performance in the current design appears to be inconsistent with the phase literature. Behavioural studies of phase using EEG and MEG have shown phase modulated effects of visual and tactile perception within the alpha band. Studies also show alpha rhythms are lateralised with stimuli processing occurring at the contralateral hemisphere in visual-spatial and tactile-spatial attention, indicated by power differences between hemispheres (Jensen & Mazaheri, 2010; Schubert et al., 2015). The use of tACS has demonstrated it acts as a causal modulator of visual and tactile processing that can suitably entrain target rhythms. In tactile perception, the phase of the tACS cycle has been shown to effect processing of stimuli when the stimulation is individualised to participants intrinsic frequency. In line with findings in visual perception, this study indicated that tACS also affects somatosensory perception by inducing phase-dependent moments of neural inhibition and excitation (Gundlach et al., 2016). However, tACS

phase-locking has been shown to occur outside the intrinsic frequency. For example, Cecere and colleagues (2015) used tACS to alter participants intrinsic alpha rhythm by +/- 2 Hz showing that perception of 1 or 2 stimuli was mediated by this change in the oscillatory frequency indicating that tACS could successfully realign endogenous rhythms above or below their average speed (Cecere et al., 2015). To what extent individualised alpha (or any frequency of interest) is required, is a matter for debate. The present study classified the alpha range as 8 - 14 Hz, this alone may be considered a methodological issue (with others arguing that the alpha rhythm falls in to a different range). Putting aside the contentious issue of alpha range, some researchers opt for a global alpha value for all participants when using stimulation techniques such as tACS. Had we elected to implement this strategy it would have removed the need for a pre-experiment (where IAFs were determined using EEG). This procedure was necessitated by literature that emphasises the requirements of individualised frequencies in the successful coupling of two weak oscillating signals (e.g. Pikovsky et al., 2001). The average value for alpha value is usually considered 10 Hz (which is the mean frequency when the alpha range is considered 8 – 12 Hz), however, given the frequency range for alpha in this study, a mean alpha frequency would have been 11 Hz, with the fastest and slowest iterations being +/- 3 Hz from this frequency. Cecere et al. (2015) appeared to demonstrate tACS phase coupling occurred +/- 2 Hz from the intrinsic frequency, thus to adopt this method either a different alpha range would need to be considered or it would need to be shown that a possible variation of up to 3 Hz between the intrinsic frequency and the tACS signal could still be successfully entrained.

Additionally, as the IAF was determined during a passive pre-experiment procedure with little or no cognitive load (i.e., more exogenous in nature than endogenous), it is possible that different attentional tasks produce different intrinsic alpha range frequencies in the tactile modality. Most evidence suggests the lateralisation seen during tactile attention, is weak or absent for exogenous attention and highly lateralised during endogenous attention. Although this lateralisation is related to power, based on the differential effects during separate types of attention, the measure of IAF may have been better suited for exogenous tactile attention only. Whether this would have altered our underlying results is unclear, however, as intrinsic

alpha frequency is not fixed and susceptible to fluctuations in a given range, it may have been helpful to determine alpha rhythms in a task-relevant fashion.

8.2 Phase effects during beta stimulation

We expected to find no effect of phase for any beta condition, due to its lack of relevance to the attentional network at the somatosensory cortex. Again, differing from our predictions, an effect of phase was found for targets cued to the contralaterally stimulated hemisphere in the exogenous task.

Typically, beta oscillations are associated with motor functioning (Jensen et al., 2002). Suppression of beta at the motor cortex has been shown to occur prior to and during movement, before an increase in power following the movement (Pfurtscheller & Da Silva, 1999). Evidence also suggests a lateralisation effect occurring in the beta-band (14–30 Hz) at the motor cortex (Jenkinson & Brown, 2011). Research indicates that this reduction in beta reflects the preparation to move (Neuper & Pfurtscheller, 2001) with lateralisation occurring when participants are aware of which hand to move, with greater beta suppression at the hemisphere contralateral to the movement hand. Faster RTs are also associated with a decrease in beta activity compared to a non-lateralized beta suppression. Beta power is shown to be at its lowest during movement execution (Kilavik Zaepffel, Brovelli, MacKay & Riehle, (2013) and is reported during stimulus-triggered movements when responding with the fingers (Gaetz, Macdonald, Cheyne & Snead, 2010), wrist (Alegre et al., 2006), feet (Pfurtscheller & Da Silva, 1999) or orally (Crone, Miglioretti, Gordon & Lesser, 1998). The beta power decrease lasts as long as the movement (Wheaton, Fridman, Bohlhalter, Vorbach & Hallett, 2009) whilst muscles are contracting (Omlor, Patino, Mendez-Balbuena, Schulte-Mönting & Kristeva, 2011). Though beta oscillations are consistently associated with the sensorimotor system, some research has also indicated a link between beta oscillations and the somatosensory domain. Beta power has been correlated with visual perception in a motion-induced blindness paradigm (Kloosterman et al.,2014) and research using monkeys indicate its involvement in decision making in both endogenous and exogenous tasks (Pesaran, Nelson & Andersen, 2008; Wimmer, Ramon, Pasternak, & Compte, 2016; Wong, Fabiszak, Novikov, Daw, & Pesaran, 2016). Several theories for the role of beta oscillations in cognition have been proposed, with various studies specifically linking

beta with endogenous processing (see Engel & Fries, 2010 for a review). Research has also indicated that inducing beta oscillations using tACS can facilitate reaction times due to the retrieval of motor tasks from memory (Krause, Meier, Dinkelbach & Pollok, 2016). One recent study indicated that tACS applied at 20 Hz over the left frontal and right parietal cortex improved performance in an attentional blink task (Yaple and Vakhrushev 2018). To sum up there is some evidence that beta oscillations may also play a role in somatosensation, most notably in the endogenous processing. However, if these effects are due to stimulation alone or can be further classified by the phase of the tACS signal is unclear. Our finding indicates that RTs during beta stimulation are mediated by phase during a tactile exogenous task.

Few studies have looked at phase specific effects of beta oscillations on the sensory cortex, however, some research has indicated phase specific effects of beta tACS over motor areas. Nakazono et. al (2016) noted a significant effect at 90° beta phase compared to 90° phase of alpha on primary motor cortex excitability. The study also revealed enhanced motor evoked potentials at the 90° beta tACS phase compared to no stimulation. Schilberg et. al (2018) also indicated a phasic link with motor evoked potentials of beta tACS however their results were only significant for participants with individualised beta below 19Hz. Using tactile stimuli in conjunction with MEG, Baumgarten and colleagues (2015) determined neuronal correlates of the time windows perceptually separating two presented stimuli. The study indicated that oscillations not only at alpha and but also beta frequencies may act as perceptual cycles in somatosensation, consistent with the theories of periodic sampling, sensory gating, and timed inhibition. Baumgarten et al. (2015) provided evidence for perceptual differences in the beta frequency range using a discrimination task, the present study provides evidence for an oscillatory relationship at a higher frequency of beta than previously determined. Whether this discrepancy reflects the attentional nature of the paradigms, as opposed to perception requires further study.

Another possible explanation for phasic effect on RTs during beta stimulation is that the phase does affect the motor response. Exogenous tasks with a SOA greater than 300 ms produce IOR and some evidence does suggest that IOR is not only related to an attention effect but is also partly due to the motor system (see Taylor & Klein, 2000). Though beta activity is generally associated with movement preparation and execution,

desynchronization of beta over the contralateral sensorimotor cortex is seen during EEG (Cassim et al., 2001). Localisation of beta band desynchronization can be slightly more posterior than beta synchronisation, suggesting that beta activity relating to motor preparation and expectation may be partially generated at regions near to somatosensory areas (Pfurtscheller et al., 1996). Taking this into account, it is possible that beta stimulation sufficiently entrained the anterior motor network during the exogenous task and the relationship between IOR and the motor system were mediated by phase with either motor preparation or execution, phasically modulating RTs. In contrast to alpha-band tACS modulation, few studies have focussed on the effects of beta rhythm and the role of phase and as such this study highlights a need for further investigation. Future research may wish to explore the effects of beta phase in somatosensory processing with or without the use of tACS. Given the relatively few number of studies reporting beta-tACS effects on reported stimulus perception, it is currently difficult to either reject or establish a causal link between the two.

Opposite to our predictions, participants showed a phase dependence to contralateral beta stimulation to cued targets during the exogenous task. Beta stimulation contralateral to cued targets during an exogenous tactile task was expected to show no effect of phase, and the results are more in line with what we expected during alpha stimulation. As stimulation was directed to the somatosensory cortex this result suggests that the phase of a 25 Hz oscillation plays some role in contralateral processing of tactile stimuli. This is despite research showing that the alpha frequency is the predominant rhythm in tactile and spatial attention. Theoretical guidelines suggest that harmonics and subharmonics of a tACS signal can also phase-lock intrinsic frequencies (Ali et al., 2013). Although no participants' IAF was measured as 12.5 Hz (which is exactly half of 25 Hz), IAF is an inherently volatile measure, not necessarily fixed and as such any value obtained cannot be said to be consistent throughout even a short period of time, or when performing the same task. Therefore, if a sufficiently high current was applied during the tACS (which, as discussed earlier, there is conflicting evidence for; see Johnson et al., 2019; Vöröslakos et al., 2018), it is possible that 25 Hz stimulation successfully phase locked subharmonic alpha activity close to the 12.5 Hz frequency. In line with this, studies using a rhythmic visual flicker at frequencies above alpha have shown that when the alpha frequency and flicker frequency have a harmonic

relationship, steady state visual evoked responses were more likely to occur in the alpha range (Herrmann, 2001). If the harmonic response found for visual rhythmic flicker is similar to the neural response produced by the tACS rhythmic signal, then the phase effect found for beta could actually be an effect on the phase of beta's subharmonic (i.e. alpha), although in theory this could be extended to other frequencies that make up multiples of 25 Hz.

8.3 The effects of the tACS protocol

Through the use of computational modelling to simulate the electric field induced by tACS we determined an effective stimulation protocol, that informed the choice of current strength and cortical regions to be stimulated. Without parallel measures of oscillatory brain activity our conclusions lie mostly under the assumption that the tACS signal did successfully phase-lock the intrinsic oscillations at the frequency applied, which some have argued may not be entirely true (see Ali et al., 2013; Reato, Rahman, Bikson, & Parra, 2010; Reato, Rahman, Bikson & Parra, 2013). Without the use of EEG or MEG, assumptions about the effectiveness of the tACS protocol and the phase or synchrony of the applied stimulation should be applied with caution. Although an online combination of EEG and tACS can be used (e.g. Gundlach et al., 2016) difficulties in the removal of the tACS induced artefacts remain (Neuling et al., 2017), recent studies do suggest more advanced techniques that may be applicable to this combined technique (see Noury & Siegel, 2018).

Another possible explanation, proposed by Helfrich, Herrmann, Engel and Schneider, 2015, is that the tACS signal imposes cross-frequency interactions that interfere with the normal network structure. Their analysis indicated that 10 Hz tACS increased phase-amplitude coupling between the alpha and gamma rhythms and a correlation between 40 Hz tACS and suppression of alpha power, in line with previous research on the alpha/gamma relationship (Helfrich, et al., 2014). This evidence for tACS effects on cross-frequency relationships points to the possibility that tACS itself may alter the intrinsic nature of the brains global functioning. Although perceptual studies using tACS have found phase specific effects, perceptual and attentional networks are likely mediated by different mechanisms and so adhere to different rules of functional connectivity. tACS at IAF for contralateral alpha stimulation to cued targets in the

endogenous task may have not displayed an effect of phase due to a lack of relevant cross-frequency interactions. Similarly, the effect of phase for beta tACS contralateral to cued targets in the exogenous task may have promoted some underlying cross frequency interactions. The general point is that in order to understand how tACS affects phase and behaviour then one must also appreciate that changes that occur within the brain when stimulation occurs may be due to both the applied frequency and its ongoing interactions with alternative frequencies. In the current design tACS was used primarily as a timing mechanism, and the phase-locking effect is presumed to merely entrain intrinsic neural populations, however regardless of the simplicity of this idea, there is evidence that tACS does interfere with neural activity other than to purely entrain it. To what extent is still to be determined, but this cannot be ignored when inferring how the brain behaves from studies that use brain stimulation methods.

tACS has also been shown to cause slight discomfort and produce visual phosphenes. Though ratings of peripheral sensations were low, participants observations were collected at the end of the session, rather than between experiments, or between blocks. This allowed for a great level of acclimatisation to the tACS sensation. Had participants been asked to report sensations more frequently it may have been possible to determine whether additional sensory interference may have been interfering with performance. A lack of findings in some conditions may be attributed to sensory interference and discomfort due to the stimulation. Applying a current below threshold would alleviate these issues, however this level of stimulation would unlikely be sufficient to phase-lock the underlying neural populations (Underwood, 2016).

Another explanation for the phase effects found for targets cued contralaterally to stimulated hemisphere during beta may be electrode placement. The montage employed in this experiment was required to stimulate only one hemisphere, target the primary somatosensory cortex with maximum intensity, whilst avoiding stimulation of the visual cortex. Using current flow modelling software electrode placement was based on this requirement. The parietal electrodes were placed at Cp3 or Cp4 and frontal electrode was placed in Fp1 or Fp2 (see Figure 8). Modelling software indicates that this arrangement would lead to the maximum current density being concentrated between the two electrodes (Faria et al., 2011). However, as the nature of tACS (when using

anodal and cathodal electrode pairs) is anti-phasic the frontal electrodes were also oscillating at the same frequency as the parietal electrodes. This leads to the possibility that phase-locking of frontal positions may have occurred and be related to the effects of phase found. Some evidence does suggest that the phase of frontocentral areas affects performance in the high alpha and low beta frequency range. Investigating the trial-by-trial influence of oscillatory phase on saccadic reaction time and discrimination tasks Drewes and VanRullen (2011) showed that the phase of ongoing pre-stimulus activity at frontocentral locations (with a slight influence from occipital areas) was associated with frequencies ranging from $11 - 17$ Hz. This finding has implications for the present study, due to both the frequency range and the montage adopted. It is possible that frontal electrode placement may have entrained similar areas as those shown to be phase dependent by Drewes and VanRullen and the effects on phase seen currently are a result of this. Notably this previous study also found that the phase effects was more prominent during a simple RT task compared to a discrimination task. This finding is analogous with the exogenous task used in our design, where we found only an effect for beta. Taken together this opens up the possibility of phase-locking due to frontal stimulation in the beta range or, as discussed earlier, a harmonic frequency of beta. At the very least, this has implications for the employment of montage design. Additionally, evidence from TMS studies suggests that cortical asymmetry may exists during visuospatial attention (Capotosto et al., 2012), therefore we cannot rule out that this exists in tactile attention. Participants whose left hemisphere were stimulated may have performed differently to those who were stimulated at the opposite hemifield with differential effects for parietal stimulation between the left and right hemisphere. A similar regional effect during tACS may interfere with tactile spatial processing. Future studies may wish to include a control montage, where alternative placements may rule out any effect of frontal stimulation or cortical asymmetry in the current experiment.

Alternatively, the lack phase effects for contralateral alpha stimulation to cued targets in the endogenous task may be because of resonance of the alpha rhythm, due to the constant addition of energy to the network that leads to a generic non-phase dependant response to cued stimuli contralateral to stimulation (see Veniero et al., 2011 for a similar idea related to rhythmic TMS). The non-stimulated hemisphere would escape this energy build-up, which could also explain (assuming phase-locking occurred

at both hemispheres) the significant phase effect found for targets cued contralaterally to the non-stimulated hemisphere in the present study. This effect would also presumably manifest itself during exogenous alpha stimulation as well as contralateral beta stimulation to cued targets and thus negate any contralateral effect of phase, however our results indicate no such effect for contralateral beta stimulation during the exogenous task.

8.4 Precision timing and post-processing

The significant pairwise comparisons found in two conditions and the resulting distributions provide evidence for a performance related phase dependence in the RTs. However, had the stimuli onset timing been more efficiently programmed, such that stimuli onset occurred at the beginning of each of the 6 phase bins, then this would have not necessitated the need to minimise variability in the timings through the removal of 10° from each phase bin. This variability may explain why significance was only found between the 60° bin and the 180° bin in both significant results. The near significance for additional bins in the alpha condition may be explained by variability in the timings, such that some of the trials may have belonged to adjacent bins. The effect of variability in timing measurements is likely to be more pronounced the faster the tACS signal, thus produce greater variability the faster the IAF. The greatest level of variability would have occurred during beta stimulation, which may also explain the lack of significance found for the bin adjacent to the phase aligned slowest bin at 300°. Assuming some trials may belong in the preceding bin, a redistribution of some of the RTs across the two bins would likely have the effect of increasing the mean RT for bins that were followed by slower bins. In the case of the significant results found presently, this would have the net effect of slowing the mean RTs in the phase bin preceding the phase aligned bin and increasing mean RT speeds for the phase aligned bin, producing a distribution that more closely followed the pattern of the tACS signal.

The lack of phase dependency for targets cued to the contralaterally stimulated hemisphere in the alpha endogenous task is in contrast to studies of tactile perception and lateralised attention affects in the somatosensory cortex. It is possible that the effect was there, but the size of the phase bins removed the ability to detect it, and the phase effect was simply averaged out. Using more phase bins may have produced more

robust differences between RTs, however, this would also have reduced the number of trials per bin and increased the chance of participant removal due to lack of trials. Whereas, using fewer phase bins would have increased the number of trials per bin, and potentially increased the number of participants included in analyses. However, this may have also made it harder to detect any effect of phase as RT distribution would be greater within each phase bin. Striking a happy medium between number of bins and number of trials per bin would likely be much easier with a sufficiently large amount of trials. As a pre-requisite for the implementation of the Chronos, no interference could occur with what methodology had already been laid out. The number of possible trials per condition was already set before the phasic element of this study was conceived, as was the random nature of the stimuli onset (by a random ISI). The duration of a tACS sessions was based on up to date recommendations and so both attentional paradigms were constrained by these time limits. Future research may consider testing just one form of tactile attention in a session, this would allow for more trials to be collected during the allotted time. If using a Chronos (or Chronos like device) the timings of stimuli presentation could be set so that stimuli onset occurs at the start of each phase bin, with a suitable buffer between each bin. This would presumably lead to less trials being removed due to slight variability in timing measurements.

By incorporating the Chronos in to the design we succeeded in extracting the phase of the tACS signal and associating the moment of stimulus presentation with the phase. Had our methods been unsuccessful it is unlikely that any significant results would have been found, rather, inaccurate timing measurements would likely manifest as no effect, equivalent to the random trial by trial variations often reported before the notion of phase effects became prevalent. Without relying on separate recording equipment (such as EEG and MEG), we have shown that improved timing measurements are a reliable means to deduce where in phase stimuli occurs during tACS presentation. However, the absence of this online neural data means that we are not able to further analyse our unexpected results and further determine what lateralisation effects or topographical activity was occurring during alpha and beta stimulation or to what level neural phase-locking occurred on a trial by trial level.

9. Conclusion

This project allowed us to successfully, through hardware and software modifications, provide a functional system that extracted phase data. The new lab set-up allows us to record and present tACS, tactile stimuli, and record response times through E-Prime with millisecond accuracy. Though some limitations are apparent, the majority of these can be addressed during the experimental design process. Once some of these concerns have been addressed stronger conclusions can be drawn about how phase affects performance during tactile attention. Regardless of the issues outlined, the present data supports the notion that both individualised alpha and 25 Hz beta tACS can be used to manipulate tactile attention and stimulus processing by phase-locking and that there is an effect of phase for both frequencies in the two different types of tactile attention.

Many of the studies that used a tACS protocol mentioned throughout this thesis could have benefitted from adopting our proposed methods to extract phase information. As shown here, the experimental paradigm itself need not be altered, and with additional settings and cleaning methods phase information would be available. This additional data could be used to provide a great deal of insight in to, not only, phase dependence of alpha frequencies and somatosensory processing, but also alternative frequency ranges, their associated modalities and cortical regions (e.g., beta and the motor cortex). With respect to the methodological issues associated with trial weighing, phase alignment, differing tACS electrode placement between sessions (and the subsequent effects these had on choice of analyses), it should be acknowledged that the experiment that the phase extraction was added to was not initially designed to determine the phase dependence of stimuli onset during tACS. The data and knowledge gained from this addition to the original study design can be considered more akin to a pilot study. Trial numbers, phase alignment, phase-binning, harmonics, electrode placement, analyses, are some elements that would require consideration in follow-up studies. Regardless of the issues discussed, and the benefit of hindsight, this study provides novel evidence for a phasic relationship between performance variability and somatosensory attention. The findings go beyond the evidence of alpha and low beta oscillations in the somatosensory cortex acting as cycles that affect perception phasically, demonstrating a phasic influence in attentional processes. Though

underpowered, this study still adds to the understanding of these mechanisms and the methods used and is likely to inform future research.

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