TMS entrains occipital alpha activity:
Individual alpha frequency predicts the strength of entrained phase-locking

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Highlights

* online, trial-by-trial entrainment of local neural synchrony in V1/V2 (rhythmic TMS)
* occipital entrainment with concurrent neural stimulation (TMS) and recording (EEG)
* 4-pulse TMS at 10 Hz yields lasting (300 ms) phase-locking at the alpha-band
* individual alpha frequency positively correlated with inter-trial phase coherence

Abstract [248/250]

Background: Parieto-occipital alpha rhythms (8-12 Hz) have been shown to underlie cortical excitability and influence visual performance. However, how the occipital cortex responds to an externally imposed alpha rhythm via transcranial magnetic stimulation (TMS) is an open question.

Hypotheses: 10-Hz rhythmic TMS can entrain intrinsic alpha oscillators in the occipital cortex.

Specifically, we predicted: (1) progressive enhancement of entrainment across time windows, (2) output frequency specificity, (3) dependence on the intrinsic oscillation phase, and (4) input frequency specificity to individual alpha frequency (IAF) in the neural signatures.

Methods: We delivered 4-pulse rhythmic TMS at 10 Hz to entrain local neural activity targeting the right V1/V2 regions while participants performed a visual orientation discrimination task. Concurrent electroencephalogram (EEG) recorded TMS-driven changes of local oscillatory activity.

There were two control conditions: arrhythmic-active and rhythmic-sham stimulation, both with an equal number of pulses and duration.

Results: The results were consistent with the first three hypotheses. Relative to both controls, rhythmic TMS bursts significantly entrained local neural activity. Near the stimulation site, evoked oscillation amplitude and inter-trial phase coherence (ITPC) was increased for 2 and 3 cycles, respectively, after the last TMS pulse. Critically, regarding hypothesis 4, ITPC following entrainment positively correlated with IAF, rather than with the degree of similarity between IAF and the input frequency (10 Hz).

Conclusions: We entrained alpha-band activity in occipital cortex for ~3 cycles (~300 ms) with our 4-pulse 10 Hz TMS protocol. IAF predicts the strength of entrained occipital alpha phase synchrony indexed by ITPC.

Keywords: Entrainment; alpha-band; transcranial magnetic stimulation (TMS); electroencephalography (EEG); visual discrimination; phosphene; V1/V2
Introduction

Brain oscillations play an important functional role in perception, attention and cognition [1–3]. Specifically, alpha-band (8-12 Hz) neural oscillations reflect ongoing sensory processing. Alpha power inversely correlates with excitability in vision [4–9], audition [10], and somatosensation [11].

In the visual cortex, the parieto-occipital alpha-band phase correlates with baseline cortical excitability (phosphene threshold) [12], gamma-band (40-100 Hz) power [13], and spiking responses [14]. In V1, alpha and gamma rhythms index feedback and feedforward processing, respectively [15,16]. Theories of oscillations postulate that alpha power reflects an active inhibition of task-irrelevant sensory signals [17–19] whereas alpha phase reflects pulsed inhibition [17,20], cyclic perceptual sampling [2] or temporal expectation [21]. Alpha oscillations are often measured but not manipulated. Here we use transcranial magnetic stimulation (TMS) to directly manipulate local alpha rhythms.

The topography of alpha power is altered by spatial attention. Typically, in endogenous (voluntary) spatial attention tasks, alpha power contralateral to the cued visual field shows reduced alpha power while the ipsilateral side shows increased alpha power [22–29]. Moreover, this lateralized alpha power modulation could reach V1 [29,30]. The parieto-occipital alpha-band activity is linked with the allocation of covert spatial attention and receives feedback control from frontoparietal cortices. The disruption of anticipatory pre-stimulus alpha rhythms on the occipital cortex brought about by transcranial magnetic stimulation (TMS) on the right intraparietal sulcus (IPS) or right frontal eye field (FEF) has been associated with deteriorated visual performance [31–33]. With Granger causality analyses applied to magnetoencephalogram data, alpha power modulation in the right FEF has been reported to predict alpha activity in V1, indicating feedback control [34]. Thus, V1/V2 may receive feedback control from FEF and IPS, two crucial nodes of the dorsal attention network.

Entrainment – the progressive phase alignment of intrinsic oscillators by external sources of rhythmic stimulation (reviews [35–37]) – has been proposed to underlie top-down modulations of
attention [38–44]. Although visual signals in visuo-occipital areas are modulated by attention [45–48], a fundamental question is whether and how the occipital cortex would respond to entrainment while participants perform a visual task, even without attention being explicitly manipulated. To address this question, we utilized a short-burst rhythmic TMS protocol along with concurrent EEG recordings [44,49], targeting V1/V2 with visual field mapping by fMRI and/or phosphene induction.

For the frequency of alpha-band stimulation, previous short-burst TMS studies used either 10 Hz [50,51] or individual alpha frequency (IAF) as the entrainment frequency [49,52]. IAF is a stable neurophysiological trait marker at rest [53], which increases with task demand [54,55] and reflects the task-required temporal integration window [56]. However, the assumption that matching the entraining frequency with IAF is important has not been directly tested in concurrent TMS-EEG studies [37]. Our strategy was to use 10 Hz as the entraining frequency and examine whether the degree of similarity between IAF and 10 Hz predicted the magnitude of phase-locked activities following entrainment.

Here we asked how the occipital cortex responds to entrainment while controlling spatial attention allocation to be distributed across the left and right visual fields, where the stimuli appeared. We aimed to entrain alpha-band synchrony in early visual areas with rhythmic 10 Hz TMS and examine the neural signatures of entrainment with EEG. We hypothesized that the occipital alpha-band activity can be entrained by targeting V1/V2 because intrinsic alpha oscillators exist in V1 [14,57] and V2 [58]. Given that V1/V2 play a pivotal role in early visual information processing, it is important to find out whether entrainment with rhythmic TMS can be established in these areas and how long such entrainment would last. We adopted arrhythmic-active TMS and rhythmic-sham stimulation control conditions, similar to previous TMS-EEG entrainment studies [44,49]. We systematically analyzed the temporal dynamics in multiple time windows during and after stimulation bursts. Although modulations of neural activity do not necessarily lead to changes in behavior, our secondary goal was to examine whether entrained alpha-band oscillations
targeting early visual cortex (V1/V2) could affect perceptual discriminability and/or criteria in a visual discrimination task.

Based on our hypothesis that occipital alpha-band activity can be entrained by targeting V1/V2 with 10 Hz TMS bursts, we tested four specific predictions characterizing TMS-driven entrainment (the first three have been previously tested in frontal and parietal locations [44,49]): (1) *Progressive enhancement of entrainment*: the strength of phase-locked activity, as measured by evoked (phase-locked) oscillation amplitude (as opposed to induced, non-phase-aligned, oscillation amplitude, [59]) and ITPC (consistency of phase alignment) [44,49], should be enhanced only after the second TMS pulse of the 4-pulse burst), because the alpha periodicity of the entraining rhythm is not defined until then. Otherwise, the results could simply reflect phase-resetting due to the first TMS pulse. (2) *Output frequency specificity*: entrained phase-locked activity should peak around the 10-Hz TMS frequency. (3) *Dependence on the intrinsic oscillation phase*: entrained activity should depend on the pre-TMS alpha phase [49]. Were the enhanced phase-locked activity due to reverberation of the imposed rhythm, rather than entrainment of intrinsic oscillators, the entrained activity should be similar regardless of pre-TMS alpha phase. (4) *Input frequency specificity*: The strength of the entrained activity should correlate with the degree of similarity between IAF and 10 Hz, given that IAF may be the intrinsic oscillation frequency of occipital alpha oscillators [49].

**Materials and Methods**

**Participants**

All 11 participants (5 female; 9 naïve to the purpose of the study; 20-47 years; M=29.9; SD=6.8) provided written informed consent. One participant's data were excluded due to excessive involuntary blinking. NYU institutional review board approved the protocol (IRB #i14-00788), which followed the Declaration of Helsinki and safety guidelines for TMS experiments [60].
The stimuli were presented on a ViewSonic P220f monitor. The screen resolution was 800(H)x600(V) at 120Hz. The viewing distance was 57 cm, set by a chin-rest. The stimuli presentation code was written in MATLAB with Psychtoolbox 3 [61,62]. To linearize stimuli contrast, the monitor’s gamma function was measured with a ColorCAL MKII Colorimeter (Cambridge Research Systems). The TMS pulses were delivered with a 70-mm figure-of-eight coil controlled by a Magstim Super Rapid² Plus¹ system. The EEG system consisted of a Brain Products actiCHamp amplifier and TMS-compatible Easycap actiCAP slim caps. See Supplementary Methods for TMS coil positioning and neuronavigation, as well as for the EEG cap layout, impedance, and event timing precision (Figure S1A).

Visual discrimination task

The participants performed an orientation discrimination task. Each trial began with a fixation period, followed by a 30-ms neutral pre-cue indicating that the target could be either in the lower left or lower right visual field with equal probability (Figure 1a). After a brief inter-stimulus interval (ISI), two Gabor patches and a response cue, indicating which patch was the target, appeared simultaneously. The Gabor patches (achromatic; 4 cpd; σ 0.42°) lasted 50 ms. Participants were asked to indicate whether the target patch was slightly clockwise or counterclockwise relative to vertical via a key press (right index finger for the ‘/’ key; left index finger for the ‘z’ key).

A central fixation cross was constantly present. Participants were asked to maintain fixation at all times and to blink after each trial. An eye tracker (EyeLink1000) ensured that fixation was within a 1.5° radius invisible circle. Trials for which fixation was broken (including blinking), from cue onset to stimuli offset, were discarded and repeated at the end of the block. The contrast level of the two Gabor patches were independently titrated before the experiment. A method of constant
stimuli (4-80% Gabor contrasts in 7 log steps) was used to obtain the contrast at which sensitivity
(d’) [63,64] reached half-of-max sensitivity of the Naka-Rushton function was defined as c50
contrast. The group average (standard deviation) of c50 contrast was 16% (4%) for both visual
fields.

During the ISI, 4 rhythmic or arrhythmic, active or sham pulses were delivered. Participants
could not tell the sound difference wearing ear plugs. The experimental design was within-subject.
The rhythmic and arrhythmic stimulations were blocked. The stimulation type (active or sham) and
the target side were randomized within blocks. The experiment consisted of 8 blocks, each
contained 64 trials, for a total of 512 trials; 128 trial repetitions per each of the 4 experimental
conditions (2 rhythmicity types × 2 stimulation types).

For the secondary goal, we found no significant effects regarding whether entrainment
modulates visual discrimination [63,64], either for visual sensitivity (d’; Figure S2) or response
criterion (c; Figure S3). The error bars represent ±1 S.E.M. corrected for within-subject design [65].

Transcranial magnetic stimulation

The TMS site for each participant was defined by retinotopy or phosphenes (see Supplementary
Methods). Figure 1c illustrates the vectors connecting the cortical and scalp sites for each
participant. All but one participant’s stimulation vector clustered around electrodes Oz, O2 and
PO4. The TMS intensity was fixed at 70% for all participants (except for one at 65% and another at
67%) of the maximal machine output, to ensure no phosphene induction during the task. The sham
control consisted of 4 pulses of pre-recorded TMS sounds played through a speaker attached to
the coil. Participants were asked to report if they saw any phosphenes at any point during the task;
they reported none.

In the rhythmic condition, the pulses were 100 ms apart, aiming to induce alpha-band
entrainment. In the arrhythmic condition, the timing of the first and last pulses of the burst were the
same as in the rhythmic condition, whereas the timing of the second and the third pulses were
randomly jittered on each trial according to a bimodal distribution synthesized from normal distributions N (±30ms, 10ms) (Figure S1B). Before and after the experiment, we verified that the registered TMS timings on the EEG achieved the expected precision (Figures S1C-D).

**EEG recordings and analyses**

Before the experiment, we recorded 2-min eyes-closed resting-state activity to define the individual alpha frequency (IAF) as the frequency corresponding to the maximum peak between 7 and 13 Hz on Welch’s periodogram [66] (MATLAB command: pwelch). The EEG recordings were digitized at 2500 Hz and down-sampled offline to 100 Hz for further analyses. All analyses were carried out with MATLAB R2017a and software packages FieldTrip [67] and Brainstorm [68]. The TMS artifacts were removed by interpolation (see Supplementary Methods). The continuous data were then segmented into epochs containing data from 300 ms before to 900 ms after the neutral pre-cue.

To assess if the neural activity was phase-locked to the entraining periodic stimulation, we calculated two indices for each of the 4 conditions: (1) evoked oscillatory amplitude (square-root of power), by averaging waveforms across trials first and then applying Morlet wavelets [59]; (2) inter-trial phase coherence (ITPC, or phase-locking value) [69] by applying Morlet wavelets to each trial and calculating their consistency with $\sum_{i=1}^{N} e^{i\theta}/N$, where $i$ denotes the trial number, $N$ the total number of trials, and $\theta$ the phase. ITPC is a ratio between 0 and 1, in which 1 indicates perfect phase alignment. For both indices, each Morlet wavelet had 5 cycles, and the frequency range was 3-50 Hz.

We analyzed the evoked oscillation amplitude and ITPC in 7 planned time windows: W0 (5-105ms, before the first pulse), W1 (105-205ms, between the first and second pulses), W2 (205-305ms, between the second and third pulses), W3 (305-405ms, between the third and last pulses), W4 (405-505ms, first cycle after the last pulse), W5 (505-605ms, second cycle after the last pulse), and W6 (605-705ms, third cycle after the last pulse). These planned time windows are consistent
with reported time intervals of entrainment effects by TMS [44,49,51]. The planned contrasts were corrected for multiple comparisons within, but not across, time windows. Within each time window, topographic analyses were corrected for multiple comparison across channels with cluster-based permutation tests [70]. According to the trial structure of the visual discrimination task, stimuli were presented in the middle of W2 (530-580 ms). Online eye tracking ensured that the trials containing eye blinking were discarded so that the analyzed EEG time windows were free of such artifacts.

Several statistical tests were performed. (1) As entrainment should lead to localized elevation of evoked oscillation amplitude and ITPC near the stimulation site, t-tests were performed by averaging the neural signatures in channels O2 and PO4 in the 10-Hz band at all time windows. (2) To explore the temporal and spectral specificity of entrainment, t-tests of the two neural signatures were performed across all time-frequency bins averaged across channels O2 and PO4. (3) To explore the topography of neural activity before, during and after entrainment, t-tests were performed across all channels in the 10 Hz band at all time windows. (4) To examine whether ITPC enhancement following rhythmic TMS depended on pre-TMS (200 ms before the first pulse) alpha phase, ITPC across participants and time points in W2-W6 were assorted into 6 equidistant 10 Hz phase bins for one-way repeated measures ANOVA and a regression analysis with a sine wave (\(y = a\sin(f^*x^*\pi/3+\phi)+c\), where \(x\) is the bin number; \(a\), \(f\), \(\phi\), and \(c\) were free parameters) [49]. (5) To examine the relation between IAF and ITPC, a linear regression was performed.

For the rhythmic-active condition, the arrhythmic-active condition is a more stringent control than the rhythmic-sham condition across all figures (Figures 2, 4-6, S4-S7) (see also [49]). Across all time windows (Figures 4-6, S4-S7), the contrasts between the rhythmic-active and arrhythmic-active conditions were significant whenever those between the rhythmic-active and rhythmic-sham condition were significant, except for evoked oscillation amplitude and ITPC in W0 and evoked oscillation amplitude in W1 (Figures S4, S5A). Therefore, we report the statistical contrast between the rhythmic and arrhythmic-active stimulation conditions.
Results

We present analyses relevant for each of the 4 hypotheses, followed by exploratory analyses regarding the temporal dynamics of the topography of the entrainment effect. Altogether both sets of results indicate successful entrainment.

(1) Progressive enhancement of entrainment. To evaluate the temporal evolution of entrainment, we obtained t-test results of the planned contrasts between rhythmic and arrhythmic-active stimulation conditions in the 7 pre-defined time windows (Materials and Methods) for evoked oscillation amplitude and ITPC (Table 1). Consistent with our prediction regarding entrained neural activity, the planned contrasts were not statistically significant 100-ms before and after the first TMS pulse (W0 and W1, p>.05), but became significantly different for the later time windows (W2, W3, and W4, p<.05). Interestingly, the occipital alpha-band stimulation enhanced phase-locked activity during the second and the third cycles after the last pulse (significant contrasts in W5 and W6, p<.05). Our entrainment effect in terms of both evoked oscillation amplitude and ITPC was long lasting (200 and 300 ms, respectively, after the last pulse of the TMS burst).

(2) Output frequency specificity of entrainment. To assess the output frequency-specificity, we conducted time-frequency analysis of phase-locked activities with frequencies ranging 3-50 Hz and time ranging 0-750 ms (Figure 2). Rhythmic-active stimulation elicited activity at ~10 Hz and its first harmonic (~20 Hz). Additionally, there were broad band responses for each of the 4 TMS pulses. Overall, the patterns for evoked oscillation amplitude and ITPC (Figure 2A,B) were similar.

(3) Dependence on the intrinsic oscillation phase. To ensure that the enhanced phase-locked activity reflected entrainment rather than reverberation of an externally imposed rhythm, we performed regression analyses on ITPC across the time windows that were significant (W2-W6) in Table 1. The pre-TMS phases (200 ms before the first 10-Hz pulse) were assorted into 6 equidistant phase bins [49]. One-way repeated measures ANOVA revealed that the ITPC values across phase bins were significantly different [F(9,45)=6.67, P<1e-5]. In this period, ITPC
depended on the phase bin with a sinusoidal trend (Figure 3A), as previously reported [49], suggesting that the phase of ongoing alpha oscillations matters. This result refutes the reverberation account, according to which ITPC would have been a flat line.

(4) Input frequency dependence of entrainment. Inconsistent with the hypothesis that the intrinsic oscillators operate at the IAF in the occipital cortex, the degree of similarity between IAF and 10 Hz did not correlate with entrained ITPC (Figure 3B). Instead, IAF directly correlated with entrained ITPC (Figure 3C). The correlations of IAF with ITPC or with evoked oscillation amplitude were not significant (Figure S8).

Topography. We explored the topography of the entrainment effects by performing topographic analysis of phase-locked alpha-band activities in each time window and assessing the temporal dynamics of evoked oscillation amplitude and ITPC. Cluster-based permutation tests [70] were used for correction of multiple comparisons. Descriptively, with the same alpha threshold for cluster-based permutation test, 10-Hz activity was initially widespread, likely due to EEG volume conduction, and progressively became more local over time. The statistically significant cluster included the frontal and occipital regions for both evoked oscillation amplitude and ITPC in W2-W4 (Figures 4, S6-S7). Note that the occipital clusters in W2-W6 (Figures 4-6, S5-S6) were more lateralized towards the stimulated (right) side. See Supplementary Results for converging evidence of lasting entrainment effects.

Discussion

This study is the first to show TMS entrainment of alpha activity in occipital cortex. We evaluated whether occipital alpha-band activity can be entrained by TMS targeting V1/V2 while participants performed a visual discrimination task. We tested the following hypotheses, based on established entrainment characteristics: (1) progressive enhancement of entrainment, (2) output frequency specificity, (3) dependence on the intrinsic oscillation phase, and (4) input frequency
specificity. Our results are consistent with the first three hypotheses, revealing that occipital alpha activity can be entrained. However, the results were not consistent with the 4th hypothesis. Instead, IAF correlated with ITPC.

Lasting alpha-band phase-locking in the occipital cortex

With a 4-pulse 10-Hz TMS protocol, we successfully entrained the right occipital cortex for 3 cycles (300 ms) after the last TMS pulse. This novel result reveals that short-burst rhythmic TMS can effectively entrain frequency-specific neural synchrony in the stimulated region. In previous concurrent TMS-EEG studies [44,49,51,71,72], short-burst rhythmic TMS elicited evoked oscillation amplitude for 1-2 cycles using an entraining frequency matching the natural oscillatory frequency of the target brain region. Note that our results fulfilled entrainment requirements and ruled out a reverberation account (as in [49]). Moreover, the evoked oscillation amplitude and ITPC at the alpha-band were not significantly different between the rhythmic and arrhythmic-active stimulation conditions in time windows W0 and W1. Therefore, it is unlikely that the lasting effects we found were due to temporal leakage of wavelets. We may have obtained longer lasting TMS-entrained duration than the study entraining right IPS [49] because our participants were engaged in a visual task, instead of in resting state. The only study evaluating TMS entrainment in occipital cortex reported not to find it [51], even though their participants were also engaged in a visual discrimination task; their 3 10-Hz TMS pulses may have been insufficient for entraining the occipital cortex.

Our results reveal that changes of two phase-locked activity measures—evoked oscillation amplitude and ITPC—show different temporal dynamics beyond the stimulation burst. ITPC lasted one more cycle than evoked oscillation amplitude (Table 1; Figures 2,5,6). As ITPC is a phase-locking activity measure that does not take amplitude into account, this finding suggests that phase could be more sensitive and informative than amplitude to index the occurrence and duration of entrainment effects in the occipital cortex.
The finding that the occipital cortex can be entrained by TMS is consistent with the notion of inter-areal entrainment as a form of neural communication whose function could be to achieve local phase alignment [36,38]. Given that visual stimuli were presented at the same timing across all conditions, the continued phase-locking in the second and third cycles after the last pulse (W5 and W6) cannot be attributed to evoked alpha activity by target onset.

**IAF directly predicts occipital entrainment**

Some studies have assumed that using IAF, the intrinsic oscillation frequency of the parieto-occipital cortex at rest, as the entraining frequency would be optimal for entrainment, but this assumption has rarely been tested [37]. Overall, our findings are inconsistent with such an assumption: The dependency of ITPC on pre-TMS phase shows that entrainment effects depend on the ongoing oscillatory state while participants are engaged in a visual task, refuting a passive reverberation account (Figure 3A). Therefore, intrinsic alpha oscillators exist in the occipital cortex. However, the parieto-occipital alpha rhythm does not necessarily possess a unitary IAF and their cortical sources can be decomposed into occipito-parietal and occipito-temporal [73] or parietal and occipital [54] components. Were IAF the intrinsic occipital oscillator frequency, smaller differences between IAF and the fixed 10-Hz entrained frequency should have led to greater ITPC. However, their null correlation did not support this hypothesis (Figure 3B). Instead, we found a direct positive correlation between IAF and ITPC (Figure 3C), which suggests that individuals with higher IAF may be more susceptible to occipital alpha-band entrainment.

**Can alpha-band occipital TMS entrainment modulate visual sensitivity (d’)?**

Our results show that visual sensitivity in a discrimination task was unaffected by the elevated alpha-band evoked oscillation amplitude and ITPC (Figures S2 and S3). Given that we only tested one specific target presentation timing, and at one TMS intensity per participant, our result does not necessarily rule out the possibility that changes in phase-locked activity level could
modulate d’. For instance, a study showed that hit rate, but not false alarm rate, in the visual field ipsilateral to TMS was significantly higher immediately after a 5-pulse alpha-band TMS over the occipital cortex than that in the sham condition [50]. Likewise, after alpha-band visual flicker, discrimination accuracy oscillates ~10 Hz [74,75]. Thus, the testing of multiple time lags at multiple intensities, as well as entraining other locations (e.g., electrode CP4), stimulating at different frequencies (e.g., theta), or manipulating spatial attention [47,48,76] could inform whether accuracy oscillates after occipital TMS entrainment and whether more elevated evoked oscillation amplitude and ITPC are required for a significant behavioral outcome.

In contrast to phase-locked analyses, some visual detection studies with trial-by-trial spontaneous oscillation analysis have revealed that pre-stimulus alpha power [77,78] and phase [79] correlate with response criterion (c) instead of visual sensitivity (d’). Further, for visual discrimination tasks, pre-stimulus alpha power correlates with confidence instead of accuracy [80]. However, note that without entrainment, trial-by-trial spontaneous pre-stimulus alpha activity is random and hence non-phase-locked across trials. Therefore, the current findings do not necessarily negate the possibility that entrainment may alter d’ following alpha-band entrainment.

Protocol considerations for future studies

An advantage of our protocol is that it has an arrhythmic-active stimulation control condition, similar to those when investigating IPS [49] and FEF [44] areas. Across time windows, using the arrhythmic-active and rhythmic-sham control conditions led to similar statistical outcomes; overall, the arrhythmic-active condition was a more stringent control condition resulting in less widespread significant statistical differences at the scalp level (Figures 2, 4-6, S4-S7).

Across the topographic analyses (except in W5, Figure 5B), the rhythmic and arrhythmic-sham stimulation conditions did not significantly differ, implying that the sound pulses alone cannot explain the entrainment of phase-locked activity. Therefore, follow-up studies may consider removing the sham conditions to increase the statistical power of different types of TMS trials.
Our 4-pulse alpha-band TMS protocol provides an interesting alternative for occipital entrainment to that of transcranial alternating current stimulation (tACS) protocols. Alpha-band tACS protocols typically involve 10-20 minutes of stimulation followed by 1-3 minutes of testing period, during which enhanced alpha-power or ITPC has been recorded with concurrent EEG and taken as evidence of entrainment [81,82] (but see alternative interpretations: [83,84]). In any case, TMS protocols [49,51], including ours, provide more focal stimulation effects than tACS and can be effectively delivered in shorter ‘bursts’ of pulses, hence pinpoint more accurately the temporal dynamics of entrainment effects, enable trial-by-trial stimulation designs and concurrent EEG recordings.

Conclusion

We have established a rhythmic 4-pulse alpha-band occipital TMS protocol for effective trial-by-trial, online brain stimulation to enable alpha entrainment in retinotopically organized V1/V2 areas. With a 300-ms entraining period, phase-locked activities persisted for ~300 ms (three 10-Hz cycles) after the last pulse. To our knowledge, this is the most effective short-burst TMS entrainment finding up to date. Moreover, we found that IAF predicts the strength of entrained phase-locking across trials (ITPC). Therefore, IAF is a key factor worth investigating in future alpha entrainment studies.
CRediT authorship contribution statement

Yong-Jun Lin: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft (with guidance and supervision from M. C.), Visualization

Lavanya Shukla: Software, Investigation, Data Curation

Laura Dugué: Conceptualization, Methodology, Writing - Review & Editing

Antoni Valero-Cabré: Conceptualization, Methodology, Writing - Review & Editing, Supervision

Marisa Carrasco: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Project administration, Funding acquisition

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Commercial relationships: none.
References


Table 1. p values of planned t-tests of evoked oscillation amplitude and ITPC at the α-band.

The frequency of interest is 10 Hz. The electrode of interest is the average of O2 and PO4 (near the stimulation loci; see Figure 1C). The time windows include the periods before (W0), during (W1-W3), and after (W4-W6) stimulation. The contrasts are significant starting from W2 until W5 or W6. *: p<.05, **: p<.01, ***: p<.001

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**Figure 1.** Experimental protocol (A), Trial structure. Each trial began with an intertrial period (ITI), followed by a neutral pre-cue to indicate the two stimulus locations. Active or sham stimulation was delivered in the interstimulus interval (ISI) between the neural pre-cue and the Gabor stimuli. The response cue indicated which Gabor was the target. Participants responded whether the target Gabor was tilted to the left or to the right with a key press. (B), Stimulation patterns & analysis time windows. The onset of the pre-cue is at t = 0. The four red lines mark the pulse timings in the rhythmic condition. The two blue lines mark the timings of Gabors onset and offset. In the rhythmic condition, the gap between pulses was exactly 100 ms. In the arrhythmic condition, the timing of the second and the third pulses was jittered (see text and Fig S1 for the probability distribution). Time window W0 is the cycle before the first pulse. Time windows W1, W2, and W3 are the cycles between pulse pairs. Time windows W4 and W5 are the first and the second cycles after the last pulse, respectively. (C), TMS loci & directions. Each cylinder represents the TMS directional vector of one participant on the MNI brain template. The cyan discs indicate the EEG electrode positions.
Figure 2. Time-frequency analysis of phase-locked activities. Colored panels are the group-averaged activities per condition (rhythmic vs. arrhythmic × active vs. sham stimulation). Gray panels are the t-statistics of condition contrasts per column or row. The precue onset was defined as t=0. The electrode of interest is the average the two channels (O2 & PO4) near the TMS loci. In the t-statistic panels, the thick black contours indicate significant time-frequency bins. In each panel, the vertical black and red lines demarcate the 100 ms time windows, blue lines the Gabor onset and offset. The red lines are also the pulse timings in the rhythmic conditions. (A), evoked oscillation amplitude. (B), ITPC. Each TMS pulse elicits broad band responses. Rhythmic-active stimulation elicits activities around 10 Hz and the first harmonic. Note that the arrhythmic-active condition is a more stringent control than the rhythmic-sham condition for the rhythmic-active condition. Note that at 10 Hz, the full width at half maximum of time and frequency for Morlet wavelets with c=5 are 0.19 and 4.71, respectively.
Figure 3. Regression analysis of ITPC in the rhythmic-active stimulation condition. The time window of ITPC is 0.205-0.705 s, the same as the significant windows in Table 1. (A), ITPC depends on pre-TMS $\alpha$-phase (200 ms before the first pulse). Each point is the average of 510 samples (10 participants x 51 time points). The error bars represent ±1 SEM. This ~1 period cyclic pattern suggests that the congruency between TMS and the phase of ongoing $\alpha$ oscillations determines the effectiveness of entrainment. (B), Difference from IAF does not predict ITPC. The absolute difference between IAF and the entraining frequency does not predict ITPC during and after stimulation. (C), IAF predicts ITPC. IAF positively correlates with ITPC during and after stimulation. See text for discussion.
Figure 4. Topographic analysis of phase-locked α-band activities in W4. The panel layout is similar to that in Figure 2. The red star symbols indicate significant channels after cluster-based permutation test for multiple comparison correction. (A), evoked oscillation amplitude. (B), ITPC. Rhythmic-active stimulation elicits widespread activation. With arrhythmic-active stimulation as control, there are significant clusters in the frontal and the occipital regions. Note that the patterns between (A) and (B) are similar.
**Figure 5.** Topographic analysis of phase-locked α-band activities in W5. See Figure 4 for panel layout description. (A), evoked oscillation amplitude. (B), ITPC. Rhythmic-active stimulation elicits widespread activation. Note that (B) continues to show significant difference between conditions whereas (A) does not.
Figure 6. Topographic analysis of phase-locked α-band activities in W6. See Figure 4 for panel layout description. (A), evoked oscillation amplitude. (B), ITPC. Rhythmic-active stimulation elicits widespread activation. Note that (B) continues to show significant difference between conditions whereas (A) does not. A significant lateralized parieto-occipital cluster exists in the rhythmic- vs. arrhythmic-active stimulation contrast.