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# Occipital alpha-TMS causally modulates temporal order judgements: Evidence for discrete temporal windows in vision<sup>☆</sup>

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## ABSTRACT

Recent advances in neuroscience have challenged the view of conscious visual perception as a continuous process. Behavioral performance, reaction times and some visual illusions all undergo periodic fluctuations that can be traced back to oscillatory activity in the brain. These findings have given rise to the idea of a discrete sampling mechanism in the visual system. In this study we seek to investigate the causal relationship between occipital alpha oscillations and Temporal Order Judgements using neural entrainment via rhythmic TMS in 18 human subjects (9 females). We find that certain phases of the entrained oscillation facilitate temporal order perception of two visual stimuli, whereas others hinder it. Our findings support the idea that the visual system periodically compresses information into discrete packages within which temporal order information is lost.

**Significance statement:** Neural entrainment via TMS serves as a valuable tool to interfere with cortical rhythms and observe changes in perception. Here, using  $\alpha$ -rhythmic TMS-pulses, we demonstrate the effect of the phase of entrained oscillations on performance in a temporal order judgment task. In extension of previous work, we 1. causally influenced brain rhythms far more directly using TMS, and 2. showed that previous results on discrete perception cannot simply be explained by rhythmic fluctuations in visibility. Our findings support the idea that the temporal organization of visual processing is discrete rather than continuous, and is causally modulated by cortical rhythms. To our knowledge, this is the first study providing causal evidence via TMS for an endogenous periodic modulation of time perception.

## 1. Introduction

A large body of literature investigates the effects of alpha amplitude on perception, linking high alpha power to high inhibition. More specifically, alpha power has been shown to increase in task irrelevant areas, whereas it decreases in task relevant areas, demonstrating its role in spatial attention (Foxye and Snyder, 2011; Kelly et al., 2006; Sauseng et al., 2005). Alpha oscillations play an even more dynamic role in the context of temporal attention, decreasing/increasing its amplitude at the moment when a target/distractor is expected (Rohenkohl and Nobre, 2011; van Diepen et al., 2015). On an even finer temporal scale we find that the phase of ongoing oscillations in the 5–15 Hz range influences perception. Busch et al. and Mathewson et al. (2009) demonstrated that the phase of occipital alpha oscillations is predictive of stimulus detection performance, implying that excitability in the visual cortex oscillates at around 10 Hz (Busch et al., 2009; Mathewson et al., 2009). These findings have been replicated several times using rhythmic en-

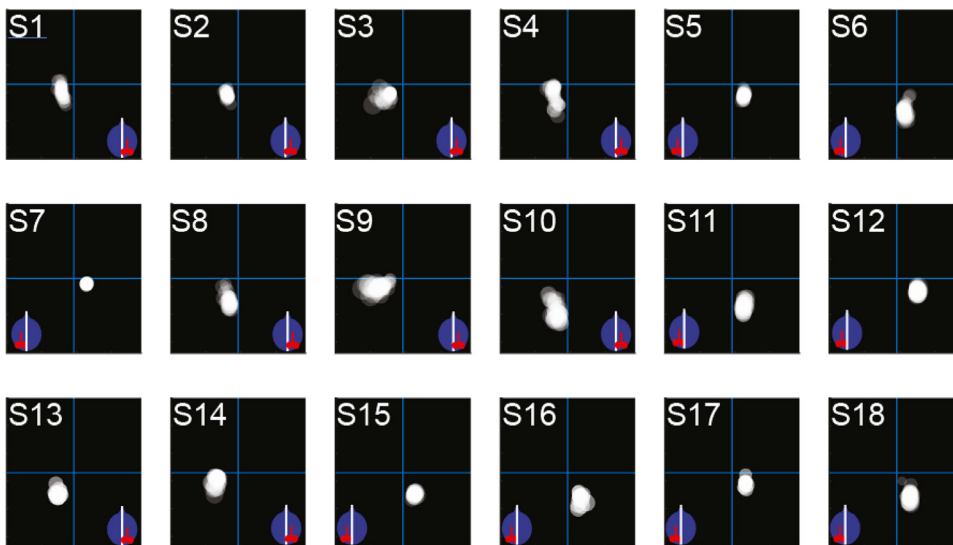
trainment at  $\sim 10$  Hz via periodic visual stimuli or alpha-TMS (Dugué and VanRullen, 2017; Mathewson et al., 2010; Spaak et al., 2014). Interestingly the ongoing fluctuations of the occipital alpha cycle also influence temporal perception periodically, giving rise to the idea that alpha implements discrete perceptual windows in vision, vaguely similar to the frames of a camera (Chakravarthi and Vanrullen, 2012; Chota and VanRullen, 2019; Ronconi et al., 2018).

More precisely the idea of a strictly discrete sampling mechanism (or "hard" version of discrete temporal perception (DTP)) states that the brain periodically divides the visual input into discrete windows or "perceptual moments" (Busch et al., 2009; Haegens et al., 2011; Lórinz et al., 2009; Samaha and Postle, 2015; VanRullen and Koch, 2003; Vijayan and Kopell, 2012). Hypothetically two stimuli that fall within one perceptual moment are perceived as occurring together whereas two stimuli falling in separate moments are perceived as occurring in succession. Recently the idea has gained renewed support by psychophysiological and electrophysiological studies linking it more di-

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**Fig. 1.** Stimulus locations for all subjects. The stimulus locations during the TMS blocks were determined during the phosphene localization. Participants had their eyes closed and received 7 TMS pulses at 20 Hz either over left or right occipital cortex. The red coil symbol over the blue head inset indicates the hemisphere of stimulation. The mouse was used by the subject to draw the outline of the perceived phosphene on the screen. Stimuli (here represented as superimposed white disks) were positioned inside the phosphene regions and scaled according to the cortical magnification factor (Horton and Hoyt, 1991). Note that due to slight changes in head position, the location of the phosphenes could change during the experiment. We re-localized phosphenes and adjusted the stimulus position at the beginning of every block.

rectly to the occipital alpha rhythm (VanRullen (2016)). It was shown that participant's individual alpha peak frequencies are predictive of performance in a two-flash fusion task (Samaha and Postle, 2015). Following these lines it was demonstrated that visuo-auditory entrainment at the individual alpha frequency  $\pm 2$  Hz could facilitate or impair performance in a temporal segregation/integration task (Ronconi et al., 2018). Further evidence comes from studies investigating the flash-lag effect (FLE), a visual illusion that has been suggested to arise from discrete sampling in the visual system (Chakravarthi and Vanrullen, 2012; Schneider, 2018). Recently we demonstrated that visual entrainment at 10 Hz leads to a periodic modulation of temporal perception in the FLE (Chota and VanRullen, 2019).

Given that alpha cycles most likely modulate cortical excitability it is critical to separate the effects of visibility fluctuations from the effects of discrete windows on time perception. The former can be thought of as a "soft" version of DTP, giving rise to fluctuations solely due to differences in excitability/visibility i.e. by suppressing one of two stimuli and therefore indirectly biasing relative timing perception. The latter can be thought of as a stricter "hard version" of DTP, implemented by discrete perceptual windows. A key aspect in the studies described above (Chakravarthi and Vanrullen, 2012; Chota and VanRullen, 2019; Ronconi et al., 2018) is that there is not only a fluctuation of perception (detection probabilities, perceived intensities), which is predicted by the soft version of DTP, but also a fluctuation of *time perception itself* (relative timing, temporal integration/segregation) which is the key prediction of the hard version of DTP. Building on top of the previous findings we set out to causally link the "hard" version of DTP closely to the occipital alpha rhythm as well as to separate observed effects from "soft" modulations of excitability. We tested this hypothesis causally by utilizing TMS to manipulate the occipital alpha rhythm and probed temporal order judgments (TOJ) at different phases of the entrained oscillation.

## 2. Materials and methods

### 2.1. Participants

25 participants (aged 18–31, 9 females) with normal or corrected to normal vision enrolled in the experiment. 7 participants had to be excluded during the first phosphene localization session because of their inability to detect TMS-induced phosphenes, leaving 18 participants for the complete experiment and the final analysis. Note that this number of subjects excluded for this reason is very common in the TMS literature. Dropout rates of 40% due to inability to perceive phosphenes (at me-

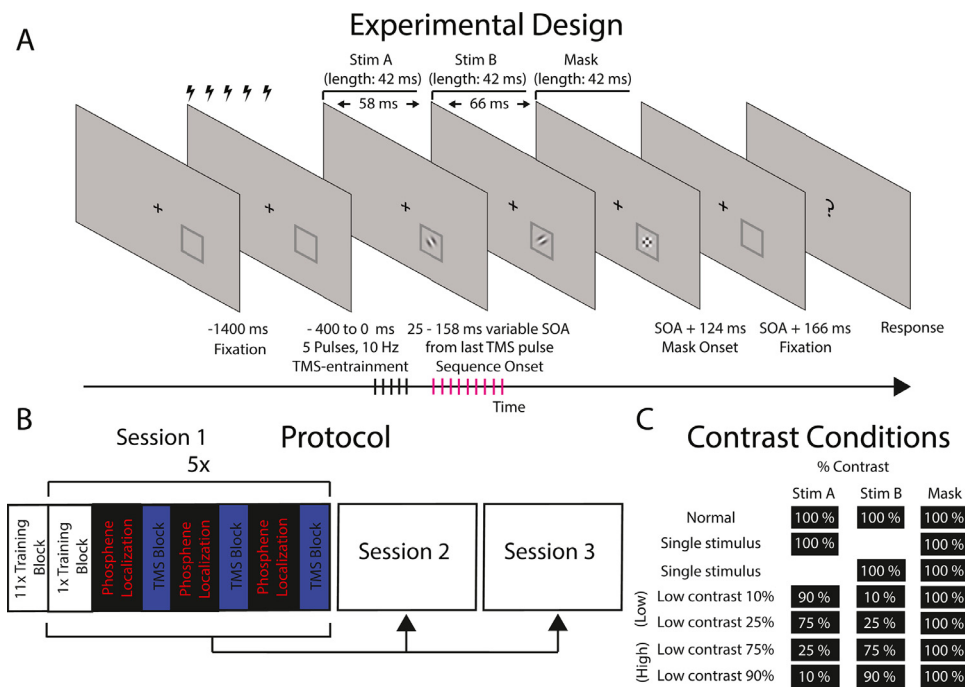
dial stimulation intensities) are commonly reported. Informed consent forms were signed before the experiment. The experiment was carried out in accordance with the protocol approved by the centre National de la Recherche Scientifique ethical committee and followed the Code of Ethics of the World Medical Association (Declaration of Helsinki).

### 2.2. TMS apparatus, parameters and phosphene localization

The TMS stimulation was performed using a Magstim Rapid<sup>2</sup> stimulator of 3.5 tesla, producing a biphasic current. At the beginning of the first session participants were tested on their ability to detect TMS-induced phosphenes. TMS stimulation was initiated at 55% of the maximum stimulator output applying 7 pulses at 20 Hz over occipital cortex. At the beginning of each phosphene localization trial participants were asked to fixate a central cross. Participants closed their eyes without changing the direction of their gaze. TMS was applied, participants opened their eyes and used the mouse to draw the outline and location of the perceived phosphene onto the screen. If no phosphene was perceived the coil position or stimulation intensity were changed manually and the procedure was repeated. When a reliable phosphene was found the coil was fixated using an armed pedestal. We successfully elicited phosphenes in 18 out of 25 subject in the right ( $N = 9$ ) or left ( $N = 9$ ) visual field (Fig. 1). Later, we used the phosphene location to place stimuli and scale them according to the cortical magnification factor (see section Stimuli below). Using a two-down one-up staircase procedure, we then determined the individual phosphene perception intensity threshold. Mean phosphene perception intensity threshold was 54.3% of maximum stimulator output. During the experimental TMS sessions we adjusted the TMS intensity to 75% of the individual phosphene perception intensity threshold (no subject reported perceiving a phosphene during the test trials).

### 2.3. Stimuli

Stimuli were presented at a distance of 57 cm with a LCD display (1920 × 1080 resolution, 120 Hz refresh rate) using the Psychophysics Toolbox (Brainard, 1997) running in MATLAB (MathWorks). Stimuli consisted of a central fixation cross (diameter =  $0.3^\circ$  v.a.), a square placeholder (black,  $4^\circ \times 4^\circ$  v.a.), Gabor patches (Stim A and Stim B) of two orientations ( $45^\circ$  and  $135^\circ$ , diameter =  $3^\circ$ , spatial frequency = 1.076 cycles/degree, std = 16 pixels/ $0.59^\circ$  v.a.) as well as a mask in the form of a plaid (diameter =  $3^\circ$  v.a.), (Fig. 2A). We included a masking stimulus in order to prevent subjects from using the persistence or the afterimage of Stimulus B to solve the TOJ task. Stimulus A will always be referring



**Fig. 2. Paradigm.** **A.** Experimental Design. Participant's initiated trials via button press while fixating at a central fixation cross. After 1000 ms 5 TMS pulses at 10 Hz were administered. We probed TOJ performance at nine time points between 25 ms and 158 ms after the last TMS pulse (SOA: last TMS pulse – first Stimulus). This was done by presenting a sequence of two Gabor patches with different orientation (Stim A and Stim B, 45° or 135°, stimulus length: 42 ms, SOA (between stimuli) 58 ms), followed by a mask to prevent stimulus persistence or long afterimages. Participants had to categorize the orientation of the second stimulus which was different on every trial. Alternatively participants could report (using a different key) a single stimulus condition where either the first or second stimulus was omitted (see C.). In the TMS Blocks no feedback was provided. **B.** Protocol. The experiment was performed on 3 separate days within 5 days. On the first session only, participants performed an extensive training consisting of 253 trials without TMS, with feedback and with a predefined stimulus location. Afterwards the main experiment was performed consisting of 5 repetitions of the Block sequence indicated in the figure.

These 5 repetitions were repeated on session 2 and 3. **C.** In order to control for an effect of TMS on the visibility of the stimuli we used 5 different contrast conditions. In a subset of trials the contrast of the stimuli in the sequence was separately reduced to either 10%, 25%, 75% or 90%. The contrast of the Mask was identical in all conditions. Additionally we included a single stimulus condition where one stimulus was omitted.

to the first stimulus of the sequence, whereas Stimulus **B** or "target" will be referring to the second stimulus of the sequence, irrespective of their orientation. During the task Stimulus **A** and **B** were presented in quick succession and participants gave Temporal Order Judgements by categorizing the orientation of stimulus **B**. Additionally we included a single stimulus condition where only one stimulus (Stimulus **A** or Stimulus **B**) was presented. Participants reported these trials using a third button. The single stimulus condition was included in order to control for the possibility that TMS entrainment could lead to a significant decrease in stimulus visibility for one of the two stimuli (**A** or **B**). In that case we would expect an increase in single stimulus reports. We will show later in the behavioral results that this was not the case.

The experiment consisted of a training condition and a TMS condition. We included a rather extensive training session in order to minimize any potential practice effects that could influence our TMS sessions and to reduce potential biases towards stimulus visibility, as we will explain later. The training condition was run with the parameters described above. The location of placeholder and stimulus sequence was fixed in the lower right or left visual field with equal probability (eccentricity 7° v.a.). The stimulus parameters in the TMS condition were identical to the training condition except for the size and position of the stimuli and placeholder which were adjusted based on the phosphene location acquired during the phosphene localization described in the previous paragraph. The location of the stimuli was chosen to be within the reported phosphene area, and as close to the position of the training stimuli as possible (Fig. 1). This ensured that stimuli in the TMS condition were not presented further than 7° visual angle from the position of the training stimuli in 96% of trials to keep training and TMS conditions comparable. The stimuli were scaled based on their eccentricity according to cortical magnification Horton and Hoyt (1991) in order to match the cortical representation of the stimulus to the actual cortical stimulation site during TMS. For example, the stimulus diameter measured 3° at 7° eccentricity and 3.78° at 10° v.a. of eccentricity. The contralateral stimulus position was determined by flipping the ipsilateral position around the central y-axis. Stimuli were equally often presented in the

left and right hemifield, following a pseudo-random sequence. Stimuli were never presented more than three times in a sequence in the same hemifield. Stimuli were presented on a gray background.

We modulated the contrast of our stimuli in a subset of trials. This was done for two reasons. First of all, it allowed us to control for the potential effects of TMS on the visibility of the stimuli. We hypothesized that any detrimental effects of TMS on visibility should be maximal when target contrast is minimal due to the introduction of noise to the neural signal, a presumed effect of TMS (Harris et al., 2008; Miniussi et al., 2010; Miniussi and Ruzzoli, 2013; Ruzzoli et al., 2010; Schwarzkopf et al., 2011). More specifically, this potential confound should manifest as a reduction in performance for stimuli that were presented on the hemifield contralateral to TMS, with high contrast stimuli being least and low contrast stimuli being most affected. As we will show later in the results section, this was not the case. Second, by having stimuli vary in contrast, we aimed to discourage participants from basing their TOJ judgments on visibility (e.g. always judging the most visible stimulus as occurring first or last), by making stimulus visibility inconsistent across trials while at the same time providing feedback. Therefore, even if the entrainment oscillatory phase should affect visibility, participants are actively encouraged to neglect visibility cues and base their decision purely on the perceived temporal order. For this second reason we decided to include several intermediate contrast conditions to better simulate perceptual ambiguity and increase the sense of unreliability of stimulus visibility. 5 Contrast conditions were used (Fig. 2C): Normal (100% contrast for **A** and **B**), low contrast 10% (Stimulus **A**: 90% contrast, Stimulus **B**: 10% contrast), low contrast 90% (Stimulus **A**: 10% contrast, Stimulus **B**: 90% contrast), low contrast 25% (Stimulus **A**: 75% contrast, Stimulus **B**: 25% contrast), low contrast 75% (Stimulus **A**: 25% contrast, Stimulus **B**: 75% contrast). Condition names (e.g. low contrast 75%) refer to the contrast value of the second stimulus of the sequence (Orientation of Stimulus **B** which had to be identified in the TOJ task) and will be used for later reference. Low percentage values represent low contrast or small difference to background luminance. The percentage values represent the Michelson contrast, defined as the

ratio between the minimal/maximal luminance value of the stimulus and the gray background ( $(\max. \text{Luminance} - \min. \text{Luminance}) / (\max. \text{Luminance} + \min. \text{Luminance})$ ).

#### 2.4. Protocol

On the first session only, participants performed an extensive pre-training consisting of 11 Training Blocks (23 trials each). Training blocks were identical to the TMS blocks with few exceptions. No TMS-pulses were applied during training and the placeholder as well as sequence presentation was in the lower right or left (equally balanced) visual field. Participants received positive or negative feedback in the form of the fixation cross turning green or red. One goal of the Training Blocks was to prevent participants from basing their TOJ judgements on the visibility of stimuli by making the contrast maximally uninformative as described above. Furthermore, the training blocks served to keep performance on a steady level by providing frequent feedback to the participant. In the training blocks 44.3% of trials were low contrast trials, 22.3% were single stimulus trials and 33.3% were normal contrast trials.

Afterwards subjects performed the main experimental procedure, which was repeated on session 2 and session 3. In the main experimental procedure participants performed 20 blocks (15 TMS blocks, 5 Training blocks) with 23 trials per block (In the last TMS block only 20 trials were collected). 3 TMS blocks were interleaved with 1 Training Block. Over the course of 3 sessions this resulted in a total of 1026 trials in the TMS and 368 trials in the training condition per subject (50% trials contralateral to TMS, 50% trials ipsilateral to TMS). Per SOA and per visual field 57 trials were collected. Before each of the 45 TMS blocks participants performed a phosphene localization to make sure that the coil position had not changed and the correct cortical area was stimulated. Trials started with the central fixation cross and the placeholder on either side of the screen (Fig. 2A). The placeholder additionally served as an attentional cue to indicate location of the stimulus sequence with 100% cue validity. This served to avoid potential spatial attentional confounds by always directing attention to the precise location of the upcoming stimuli. Participants initiated the trial via button press. 1000 ms after the button press 5 TMS pulses (100 ms between pulses) were administered over the course of 400 ms. Starting with the last TMS pulse, after a variable delay (25–158 ms in steps of 16.7 ms) the stimulus sequence was presented inside the square placeholder. The orientation of Stim A and Stim B was pseudo-randomly chosen every trial (45° and 135° or vice versa). The presentation length for Stim A, Stim B and the mask was 42 ms each. The ISI between Stim A and Stim B was 16 ms. The ISI between Stim B and the mask was slightly longer with 24 ms. During Piloting we observed a forward and backwards masking effect on the second stimulus which we compensated by shifting the onset of the mask to a later time-point. This was done to equalize visibility between Stim A and Stim B which we verified during piloting in 4 subjects. After a delay of 1000 ms participants reported either the orientation of Stimulus B (arrow key left or right) or reported perceiving a single stimulus (arrow key up). Trials were randomly chosen from the normal condition (74.27% probability) [Stim A (normal contrast), Stim B (normal contrast), Mask], low contrast condition (17.35% probability) [Stim A (low contrast), Stim B (low contrast), Mask] or a single stimulus condition (8.3% probability) [Stim A (normal contrast), omitted, Mask] or [omitted, Stim B (normal contrast), Mask] (See Fig. 2C). During the phosphene localization and the experiment, the participants head was fixed between a chinrest and the TMS coil, leading to a stable position. In total, a maximum of 2215 TMS pulses were administered per session.

We did not measure participants gaze position during the experiment. Participants were frequently reminded to keep fixation on the fixation cross, especially during the phosphene localization procedure. While participants could have occasionally shifted their gaze away from the fixation cross it is unlikely that this could give rise to oscillatory modulations of performance.

#### 2.5. Data analysis

We analyzed the data collected during the training blocks and the TMS-blocks separately. The 11 pre-training blocks at the beginning of session 1, which were included to prevent visibility biases, were not included in the analysis because of potential practice effects. Only training blocks that were collected during the main experimental procedure (interleaved with the TMS blocks) are included in this analysis ( $3 \times 5$  blocks of 23 trials per subject).

Our experiment consists of a mixture between a categorization task (two stimulus trials) and a detection task (single stimulus trials). Our reports of performance in the normal contrast as well as the low contrast conditions refer to the categorization performance  $p(\text{correct categorization of second stimulus} | \text{two stimuli presented})$  whereas performance in the single stimulus conditions refers to detection performance  $p(\text{one stimulus reported} | \text{one stimulus presented})$ . Performance in the detection task is sometimes reported as sensitivity ( $d'$ ). We calculated  $d'$  by comparing the proportion of single-stimulus responses  $p(\text{one stimulus reported} | \text{one stimulus presented})$  to the proportion of single-stimulus responses in the normal contrast condition  $p(\text{one stimulus reported} | \text{two stimuli presented})$ .

#### 2.6. Training blocks mean performance

Due to the significantly smaller number of trials collected in the training blocks compared to the TMS-blocks we did not analyze the time course of TOJ performance during training but looked at the overall performance for all 6 conditions (*normal contrast* condition, 4 low contrast conditions, *single-stimulus* condition). The 4 low contrast conditions (Fig. 2C) were merged into two groups based on the contrast of the stimulus B (target), separating them into a *low target contrast* group (Stimulus B with contrast values of 10% and 25%) and *high target contrast* group (Stimulus B with contrast values of 75% and 90%). Note that the major aim of including several fine-grained contrast conditions in the first place was to counteract potential visibility biases, by preventing subjects from relying on visibility instead of TOJ. The separation based on Stimulus B contrast was done to control for possible effects of TMS on Stimulus B (target) visibility which should be maximal for the low contrast stimuli (for which orientation was reported) in the *low target (Stim B) contrast* group. Mean performance was averaged within each subject for 1. *normal contrast trials*, 2. *low target (Stim B) contrast trials* (contrast values of 10%, contrast values of 25%), 3. *high target (Stim B) contrast* (contrast values of 75%, contrast values of 90%) and 4. *single stimulus* conditions separately. Mean performance in the categorization task was statistically analyzed using a 3-way ANOVA with the factors TMS (Training/TMS), Hemifield (contra/ipsi) and contrast (normal, low target, high target). Mean performance in the detection task was statistically analyzed using a 2-way ANOVA with the factors TMS (Training/TMS) and Hemifield (contra/ipsi) (see results Section 3.2.4).

#### 2.7. TMS blocks

##### 2.7.1. Analysis of mean performance

The analysis of the mean performance for the 6 conditions (*normal contrast* condition, 4 low contrast conditions, *single stimulus* condition) was done similarly to the analysis in the previous section by merging low contrast conditions into 2 groups (*low target contrast*, *high target contrast*). For the low contrast conditions we acquired 178 trials (*low target contrast* and *high target contrast*) per subject. For the *normal contrast* and the *single stimulus* conditions 762 and 86 trials were collected per subject. Mean performance in the categorization and detection tasks was statistically analyzed using a 3-way (categorization) and 2-way (detection) ANOVA described in the previous paragraph (see results Section 3.2.4) as well as a T-test, directly comparing differences in mean categorization performance between low target contrast and high target contrast trials (see results Section 3.1.1).

### 2.7.2. Analysis of TOJ time-series

For our main results, the effect of TMS on subsequent TOJ performance was analyzed as a function of the delay between the last TMS-pulse and presentation of the first stimulus of the sequence. Since the other contrast conditions only served to ensure that participants engaged in unbiased TOJ judgments only the normal contrast condition (74.27% of trials) was included in this analysis. We removed all responses where subjects erroneously reported single stimuli (5.4%), counting only left/right responses (making chance performance 50%). We probed TOJ performance at 9 time points (SOA's) after TMS (25 ms, 41.7 ms, 58.3 ms, 75 ms, 91.7 ms, 108.3 ms, 125 ms, 141.7 ms, 158.3 ms). On average 713.83 trials (16.02 SEM) measuring categorization performance (excluding single stimulus reports) in the normal contrast conditions were collected per subject. Performance (Hits/Total number of trials) was averaged within each subject at each of 9 SOA's for stimuli presented contralateral and ipsilateral to TMS respectively. The resulting time series were normalized by subtracting the mean and averaged over subjects, resulting in a grand average contralateral (contraTMS) and ipsilateral (IpsiTMS) time series. In an additional analysis the individual 133 ms long TOJ time-series were subtracted on a subject-by-subject basis in order to calculate the difference waves between the two conditions. These difference waves were averaged resulting in the grand average difference wave (contraTMS minus IpsiTMS). Time-series were zero-padded to a length of 6 times the original window length ( $6 \times 133.3$  ms) and analyzed in the frequency domain using FFT (frequency resolution 1.1 Hz). 9 SOA's at 60 Hz allowed for a Nyquist frequency of 30 Hz. The complex FFT coefficients were squared to obtain oscillatory power at each frequency. To statistically test if the time-series contain significant oscillatory power we calculated 1.000.000 surrogates by shuffling the SOA-labels between trials for every subject, and repeating all analysis steps for each surrogate. The original power-spectrum was then compared to the surrogate distribution and p-values were corrected for multiple comparisons using the False Discovery Rate. The FFT revealed a peak at 10 Hz for contraTMS and a peak at 7.8 Hz for the IpsiTMS condition. For the phase analysis, we therefore decided to extract individual phase angles from the center frequency 8.9 Hz component of the FFT of normalized contraTMS and IpsiTMS time-courses as well as the contraTMS minus IpsiTMS difference wave. Individual contraTMS and IpsiTMS phase angles were subtracted (contra minus Ipsi, pairwise subtraction) to investigate the phase relationship in individuals. Rayleigh's test for non-uniformity was used to statistically test if individual phases were significantly coherent.

## 3. Results

### 3.1. Main findings

In this study we seek to provide causal evidence for the hard theory of discrete perception. Specifically, we aim to show that discrete perception entails a periodic compression of time information. Our hypothesis therefore states that within a perceptual moment, perception of the temporal order of events is impaired. To test this hypothesis we entrained participants' alpha oscillations using 10 Hz-TMS over early visual areas. We probed TOJ performance, an index of perception of relative timing, at different phases of the entrained oscillation after stimulation. Because TOJ can be performed even if only one of two stimuli can be categorized, our measure of time perception is robust with regard to periodic fluctuations of visibility, as these should only affect one stimulus at a time. A significant oscillatory component at 10 Hz at the entrained location (as measured by a frequency analysis on the average TOJ time-course) would suggest a rhythmic modulation of time perception.

#### 3.1.1. General behavioral performance

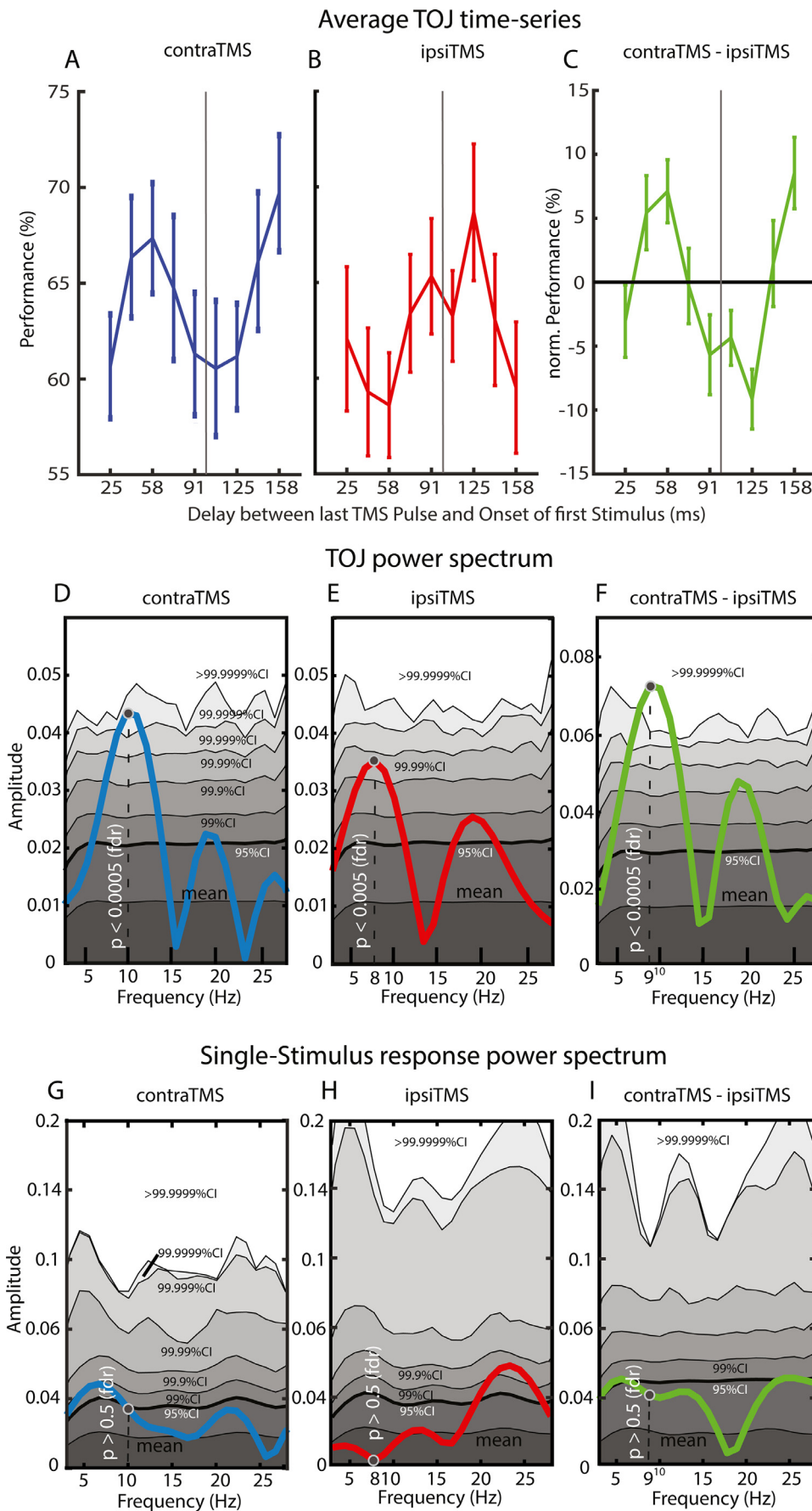
Mean categorization performance (TOJ) in the normal, low contrast and detection performance in the single stimulus conditions was compared between Training blocks (no-TMS) and TMS blocks. During

Training the mean categorization performance across subjects and visual fields was 63.14% ( $\pm 3.5\%$  SEM) for the *normal condition*, 56.4% ( $\pm 4.4\%$  SEM) for the *low target contrast* condition and 63.1% ( $\pm 3.4\%$  SEM) for the *high target contrast* condition. Sensitivity was  $d' = 0.69$  ( $\pm 0.117\%$  SEM) for the *single stimulus* condition. In the TMS blocks the mean TOJ categorization performance across observers in the *normal contrast* condition was 64.4% ( $\pm 2.6\%$  SEM) for stimuli contralateral and 62.4% ( $\pm 2.5\%$  SEM) for stimuli ipsilateral to the cortical entrainment site. For the *single stimulus* condition sensitivity  $d'$  was 1.61 ( $\pm 0.083\%$  SEM) for stimuli contralateral and  $d' = 1.59$  ( $\pm 0.106\%$  SEM) for stimuli ipsilateral to the cortical stimulation site. In the *low target contrast* condition average categorization performance was 63.6% ( $\pm 3.3\%$  SEM) for the contraTMS condition and 57.4% ( $\pm 3.7\%$  SEM) for the IpsiTMS condition. Last, in the *high target contrast* condition categorization performance was 65.8% ( $\pm 3\%$  SEM) for the contraTMS and 62.1% ( $\pm 1.8\%$  SEM) for the IpsiTMS condition. Comparable performance in high and low target contrast conditions would indicate that subjects utilized both stimuli equally to perform TOJ. Statistical comparisons of high and low target trials confirmed that performance did not differ between these types of trials during TMS (T-test: contraTMS  $p = 0.58$ , IpsiTMS  $p = 0.24$ ) and similarly during Training (T-test: contra  $p = 0.31$ , Ipsi  $p = 0.46$ ). In addition we compared performance in these two types of trials in the context of a 3-way ANOVA (with factors *TMS*, *hemifield* and *contrast*, see results [Section 3.2.4](#)) finding no significant difference. These results suggest that participants were indeed able to use any of the two stimuli to perform TOJ.

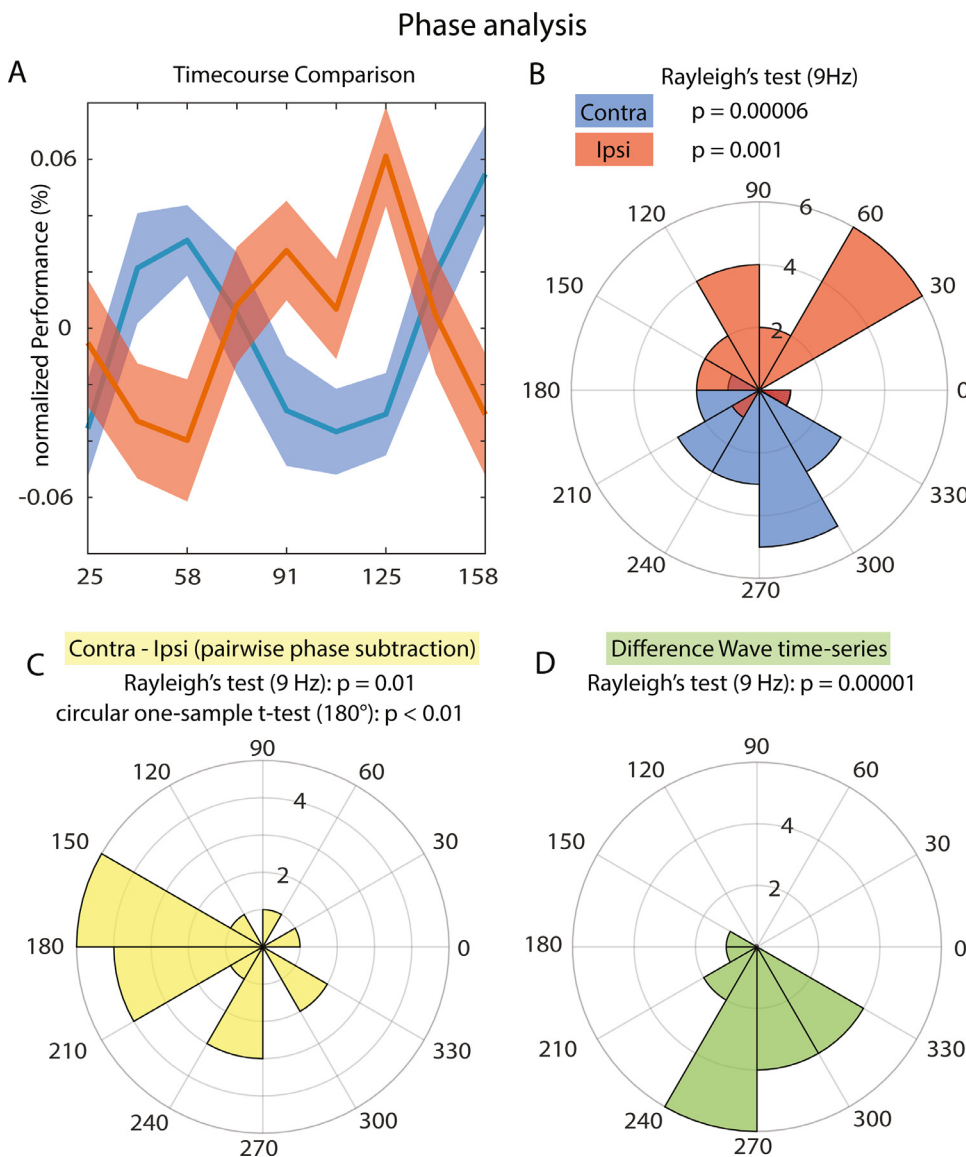
We calculated  $d'$  by comparing the proportion of single-stimulus responses in the single-stimulus TMS condition (hits: 51.9%) to the proportion of single-stimulus responses in the normal contrast condition (false positives: 5.4%). As mentioned before sensitivity was  $\sim d' = 1.6$  for contra and ipsilaterally presented stimuli indicating that participants were reasonably well able to differentiate between 2 stimuli and 1 stimulus conditions. Furthermore, the single stimulus report rate of 5.4% in the normal contrast condition indicates that participants perceived the 2 stimuli in the vast majority (94.6%) of normal contrast trials. Only these trials were included in the time-series analysis in the first [Section \(3.1.2  \$\alpha\$ -TMS leads to periodic modulation of TOJ performance\)](#). It is noteworthy that participants seemed to apply a relatively conservative criterion on reporting single stimulus trials. We discuss this further in [Section 3.2.1](#).

#### 3.1.2. $\alpha$ -TMS leads to periodic modulation of TOJ performance

In order to verify that TOJ performance was rhythmically modulated by  $\alpha$ -TMS we calculated the average TOJ time-series over individuals. This was done by calculating the average categorization performance  $p(\text{correct} | 2 \text{ stimuli})$  for each of the 9 SOA's. Performance was analyzed separately for sequences presented contralateral (contraTMS) and ipsilateral (IpsiTMS) to  $\alpha$ -TMS. Only normal contrast trials (74.27% of all trials) were included in this analysis, in order to discard any possible influence of contrast differences on stimulus visibility. For this analysis we excluded trials in which participants reported only a single stimulus: when both stimuli are visible, we can reasonably assume that subjects' reports reflect their perceived temporal order, as per the task instructions. TOJ time-series were normalized by subtracting the individual mean before applying the FFT. [Fig. 3A](#) shows the original, un-normalized time-series. Initial inspection of the time course for contraTMS indicates a strong oscillation in the TOJ performance lasting for more than 158 ms. To quantify this effect, we performed a frequency analysis on the average TOJ-time-series. The resulting power spectrum revealed a dominant oscillation at 10 Hz ([Fig. 3D](#)). For statistical validation of this peak we created 1.000.000 surrogates by shuffling the 9 SOA-bin labels within subjects and recalculated the power spectrum of the resulting TOJ time series. P-values were computed as the percentile of the mean power values within the bootstrapping distribution. This allowed us to test the null-hypothesis that the power spectrum of the average TOJ time-series does not show a peak at a specific frequency.



**Fig. 3. Main findings.** **A.** Average TOJ time-series ( $N = 18$ ) for stimuli presented contralateral to TMS. Error bars represent standard error of mean. The vertical gray line indicates the hypothetical time point of the next TMS pulse if the entraining sequence had continued. **B.** TOJ time-series for ipsilateral stimuli. **C.** Difference wave between contraTMS and ipsiTMS time series. **D.** Power spectrum of the group-average contraTMS time-series. We compared the peak at 10 Hz to a surrogate distribution (1,000,000 surrogates) which revealed a significantly higher power at this frequency compared to other frequencies after correcting for multiple comparisons ( $p = 0.00006$ , FDR-corrected:  $p = 0.005$ ). Colored areas: Dark gray: Mean of the surrogate distribution; Light gray: 95% Confidence Interval; 99% CI; 99.9% CI; 99.99% CI; 99.999% CI; 99.9999% CI; White:  $>99.9999\%$  CI. **E.** Power spectrum of the group-average ipsiTMS time-series. We observed a relatively strong oscillatory component at around 8 Hz. Analogous to (D) we analyzed oscillatory power at 8 Hz and found significantly more power compared to the surrogate distribution ( $p = 0.00038$ , FDR-corrected:  $p = 0.0039$ ). **F.** Power spectrum of the group-average contraTMS minus ipsiTMS time-series. Oscillatory peak was at 9 Hz and showed significant power ( $p < 0.0005$ , FDR-corrected:  $p = 0.0005$ ). Note that 9 Hz oscillatory power was 71% higher in amplitude compared to the 10 Hz oscillation in the contraTMS condition, indicating an anti-phasic relationship. **G,H,I.** Same as D,E,F but for the time-series of single-stimulus reports ( $p(\text{single-stimulus report} \mid 2 \text{ stimuli})$ ). No significant oscillatory peak was observed in contraTMS or ipsiTMS conditions and neither in contraTMS minus ipsiTMS time-series compared to the surrogate distribution at our frequencies of interest (5–15 Hz, FDR-corrected:  $p > 0.5$  respectively). Note that some peaks in the graph extend above the 95% CI (e.g. G at 7 Hz) but are not reported because they did not stay significant after correction for multiple comparisons.



**Fig. 4.** 9 Hz Phase Analysis. **A.** Time Course of normalized contraTMS (stimulated hemifield) and ipsiTMS (non-stimulated hemifield) for direct comparison. Note the clear antiphasic relationship between the two. **B.** 9 Hz phase angles of contraTMS (blue) and ipsiTMS (red) time-series. Rayleigh's test reveals significant phase clustering for both conditions. **C.** Pairwise subtraction of 9 Hz contraTMS minus ipsiTMS phase angles (phase domain difference). Phase angles were significantly clustered around 180° indicating an antiphasic relationship between individual contraTMS and ipsiTMS time-series. **D.** 9 Hz Phase analysis of the difference wave (contraTMS minus ipsiTMS, time domain difference). Phase angles were significantly clustered.

The TOJ time-series power at 10 Hz was significantly higher compared to the surrogate distribution (Fig. 3D, 10 Hz:  $p = 0.00006$ , FDR corrected). We analyzed the ipsiTMS TOJ time-series in an identical fashion. Initial inspection indicated a slightly weaker oscillation peaking at a lower frequency of 8 Hz (Fig. 3B). The frequency analysis and statistical analysis of the ipsiTMS time-series showed significantly more power compared to the surrogate distribution (Fig. 3E). Power peaked at 8 Hz ( $p = 0.0039$ , FDR corrected) but was still significant at the entrainment frequency of 10 Hz ( $p = 0.015$ , FDR corrected). Additionally, a visual inspection of the individual TOJ time-series revealed clear peaks in 16 out of 18 subjects in the 6.6 to 12.2 Hz range (mean 9.8 Hz, SEM  $\pm 0.51$ ) verifying that our main effect is not driven by a few subjects with strong phasic modulation.

To test if the oscillation in the ipsiTMS time-series is caused by a non-specific effect of TMS on both hemifields, we tested whether oscillations in contra and ipsilateral TOJ time-series were consistent in phase. We therefore subtracted ipsiTMS time-series from the contraTMS time-series for each subject individually. Should both fluctuations in the time-series be caused by the same non-specific effect of TMS they should cancel out and the resulting contraTMS minus ipsiTMS time-series should show reduced amplitude. On the contrary, the resulting difference wave showed markedly higher fluctuations (71% increase in range) compared to the contraTMS time-series. By performing another frequency analysis, this

time on the contraTMS minus ipsiTMS time-series, we could attribute this increase to a strong oscillatory component in the 9 Hz range (Fig. 3F, 9 Hz:  $p < 0.00005$ , FDR corrected). The increase in oscillatory power in the difference wave is likely a result of subtracting two oscillations that share a common (or neighboring) frequency but are in anti-phasic relationship.

### 3.1.3. Alpha phase analysis

To test if the oscillations observed in both hemifields are in anti-phase we analyzed the 9 Hz phase angles of the ipsiTMS and contraTMS time-series (Fig. 4A) as well as the difference wave across subjects. Since our main effects are based on frequency spectrum of group average time-series this analysis also serves to exclude the possibility that a few subjects with strong phasic effect were driving our main results (Fig. 3). The frequency of 9 Hz was chosen because it lies right within the frequency ranges of the contra and ipsilateral peak components and both time series show highly significant power at this frequency (contraTMS:  $p = 0.00017$ ; ipsiTMS:  $p = 0.0039$ ). Additionally we performed a pair wise subtraction of the individual contraTMS and ipsiTMS phase angles (phase domain difference) to test if a possible anti-phasic relationship is visible on the single subject level. The complex FFT coefficients at 9 Hz were extracted to calculate individual phase angles (Fig. 4B). We then compared these angles using Rayleigh's test



for non-uniformity testing the null hypothesis that the phase angles are randomly distributed. Phase angles were significantly clustered in ipsilateral as well as contralateral TOJ time-series (Fig. 4B, contralateral:  $p = 0.000062$ , ipsilateral:  $p = 0.0017$ ). We estimated the mean phase angles to be  $273^\circ$  for contraTMS and  $89^\circ$  for ipsiTMS time-series at  $t = 0$ . We were further interested in the phase relationship between 9 Hz oscillations in contraTMS and ipsiTMS time-series (time domain difference). To test the two sets for phase opposition we subtracted the phase angles for each subject and performed Rayleigh's test for non-uniformity on the resulting phase differences. Phase difference angles were significantly clustered at  $180^\circ$ , indicating anti-phasic oscillations at opposing hemifields (CI lower bound:  $120.5^\circ$ , upper bound:  $199.8^\circ$ , mean:  $160.2^\circ$ ,  $p < 0.01$ ). The difference wave was analyzed in an identical fashion as contraTMS and ipsiTMS time-series, showing highly significant 9 Hz phase clustering ( $p = 0.000001$ ).

### 3.2. Experimental controls

TMS could potentially lead to a periodic reduction in stimulus detection which might explain fluctuations in TOJ performance, a confound that we aim to exclude. The proportion of "single-stimulus" responses is an indicator of subjects' ability to discern the presence of two stimuli, and therefore to perform genuine temporal order judgments rather than base their responses on the most (or only) visible stimulus. We use our measures of single stimulus perception 1. to isolate trials in which participants had a clear percept of two stimuli (results Section 3.1.1). 2. to control for periodic fluctuations of stimulus detection (results Section 3.2.1) and 3. to control for potentially detrimental phase-independent effects of TMS on stimulus detection (results Section 3.2.2).

Furthermore, we utilize our low contrast conditions to control for effects of reduced orientation categorization. Importantly our TOJ task should naturally be resistant to fluctuations in orientation categorization caused by phase, since only one stimulus has to be categorized in order to solve the task. In addition, we argue that if excitability fluctuations would modulate orientation categorization, this should manifest as a 20 Hz and not a 10 Hz modulation of our TOJ time course (see results Section 3.2.3). And finally we use the different contrast conditions to help us estimate whether excitability fluctuations that partially impair orientation categorization of a single stimulus (contrast asymmetry) could lead to a decrease in TOJ performance (see results Section 3.2.4). These conditions also verified that participants were using both stimuli to solve the TOJ. Excluding stimulus detection as well as contrast asymmetries and fluctuations of orientation categorization as possible causes for TOJ performance fluctuations, leaves only modulations of time perception as plausible cause for our main findings.

#### 3.2.1. Control for periodic modulation of single stimulus responses

As a control analysis we investigated if  $\alpha$ -TMS leads to a periodic modulation of the ratio of single stimulus responses. To exclude the possibility that certain phases of the entrained alpha oscillation led to perceptual occlusion of one or both stimuli, and in turn might periodically modulate the ability to perform temporal order judgments, we repeated the analysis presented in results Section 3.1.2, this time on the ratio of single stimulus responses  $p(\text{single-stimulus report} \mid 2 \text{ stimuli})$ . This ratio can be considered a measure of excitability/visibility as it indicates how often participants reported a single stimulus when actually two stimuli were presented, thus presumably missing one stimulus due to occlusion. All analytic and statistical parameters were kept identical. The frequency analysis of contraTMS and contraTMS minus ipsiTMS revealed no peaks in oscillatory power that were significantly higher compared to the surrogate distribution (Fig. 3G,I; 5 to 25 Hz:  $p > 0.5$  respectively, FDR corrected). We observed a significant peak at 24 Hz in the ipsiTMS time-series (Fig. 3H;  $p = 0.048$ , FDR corrected). Since this oscillatory component was far away from our frequency of interest 10 Hz and not significant in any other control condition, we did not investigate it further.

As quickly mentioned before subjects applied a relatively conservative criterion when reporting single stimulus trials evident in only 51.9% of single stimulus responses in the single stimulus condition. We believe that this might be due to the fact that the perception of two stimuli without perception of their temporal order is perceptually similar to perceiving a single stimulus. During training subjects therefore might have attempted to report single stimuli, received negative feedback and adjusted their criterion. In the context of our control condition this means that our single stimulus response analysis most likely reflects mostly trials that were reported with high confidence. As there is no reason to believe that these trials should not reflect potential periodic modulations (a confound we exclude successfully) we believe that this poses no problem for the validity of our control analysis. That being said we cannot completely exclude that participants occasionally perceived only single stimuli due to phasic inhibition. Presumably the number of these trials should be low since stimuli were presented at full contrast and would present very salient events when presented in isolation. It has not yet been demonstrated that inhibitory alpha phase could perceptually hide full contrast stimuli of this size. Given that single-target reports were not modulated at 10 Hz we conclude that modulations of excitability cannot explain the modulation of TOJ performance.

#### 3.2.2. General effect of TMS on single stimulus responses

Next, we also investigated the relative number of single stimulus responses  $p(\text{single-stimulus report} \mid 2 \text{ stimuli})$  for all conditions. We hypothesized that if TMS leads to an occlusion of a single stimulus this should be apparent in an increase in the ratio of single-stimulus responses. Moreover, this should only be the case for stimuli presented contralateral to TMS. The repeated measures ANOVA revealed a main effect of contrast ( $F(2,34) = 40.72$ ,  $p < 0.005$ ) and of TMS ( $F(1,17) = 5.67$ ,  $p = 0.029$ ) but not of hemifield ( $F(1,17) = 0.008$ ,  $p = 0.929$ ). Post hoc tests revealed significantly different ratios of single stimulus reports between all contrast conditions (Normal contrast: 5.4%, high target contrast: 10.9%, low target contrast: 28.8%  $F = 24.9$ ,  $p < 0.05$  respectively). We believe that these differences are explained by the visual mask following the second stimulus. The mask likely exerts a stronger effect on stimuli that are closer in time and have a lower contrast, occasionally leading to the disappearance of the low contrast second stimulus. Importantly however any mask-induced effects on stimulus categorization performance, periodic or not, should be evident in differences in TOJ performance between low contrast and high contrast trials, which we fail to find (see first ANOVA Section 3.2.4 and T-test Section 3.1.1). In addition we found significantly higher ratios of single stimulus reports in the TMS condition compared to training ( $F = 5.67$ ,  $p = 0.29$ ), potentially caused by unspecific effects of TMS on participants single stimulus report criterion, due to their knowledge of being stimulated. Importantly however no interaction effect between TMS and hemifield ( $F(1,17) = 2.65$ ,  $p = 0.12$ ), TMS and contrast ( $F(2,34) = 1.08$ ,  $p = 0.35$ ) or TMS, contrast and hemifield ( $F(2,34) = 3.25$ ,  $p = 0.051$ ) was found, indicating that TMS did not lead to a stimulation-location specific higher number of single stimulus responses.

#### 3.2.3. Control for peak/trough and equilibrium configurations

As briefly mentioned previously, even if both stimuli have been detected, failure to categorize the orientation of one (or both) of the stimuli could potentially bias TOJ performance in a phase-dependent manner. On the other hand, this situation might arise if the two stimuli fall evenly on the left and right side of an excitability peak or trough (an "equilibrium configuration") and both suffer from a relative decrease in orientation categorization compared to the excitability peak. These 2 cases (peak/trough configurations and equilibrium configurations) might affect orientation categorization to the same extent, in which case we would not expect to see resulting fluctuations in performance. If they differ however, e.g., if the orientation of stimuli in equilibrium states is harder to categorize, then we would expect a drop in performance two times per cycle (when the stimuli fall on both sides of the peak

and again when they fall on both sides of the trough). Importantly, this should manifest not as a 10 Hz oscillation but as a 20 Hz oscillation in our behavioral time-course. In fact we did observe a slight modulation at 19 Hz in our behavioral time-course (Fig. 3). However this oscillatory component did not reach statistical significance ( $p = 0.11$ ). We conclude that while excitability fluctuations might slightly modulate performance (at 20 Hz) they cannot explain the 10 Hz peak, which is better explained by discrete temporal windows in vision.

### 3.2.4. General effect of TMS and contrast asymmetries on performance

To investigate if TMS or contrast asymmetries had a detrimental phase-independent effect on performance when stimulus visibility is low, both TMS blocks and Training blocks were analyzed using two repeated measures ANOVA's with factors TMS (TMS versus Training), hemifield (contra versus ipsilateral to TMS) and contrast (normal, low target contrast, high target contrast) for the 3-way ANOVA, and only including single stimulus trials for the 2-way ANOVA. We were particularly interested in 1. verifying that TMS did not cause any phase-independent decreases in task performance, as this would indicate that changes in excitability (here not via phase fluctuations but e.g., via increases in alpha power) could potentially deteriorate orientation categorization and 2. to verify that asymmetries in stimulus contrast did not impair TOJ performance, as effects of rhythmic entrainment might lead to asymmetries in orientation perception and thus might influence TOJ performance.

We hypothesized that if TMS had a phase-independent detrimental effect on orientation categorization, this should be reflected in average performance differences (collapsed over SOA's) when comparing TMS and Training (no-TMS) trials. Furthermore, these differences should be even more pronounced in low contrast conditions, where the target visibility is closer to the visibility threshold. Notably occipital alpha power has been shown to predict cortical excitability (Lange et al., 2013; Romei et al., 2008). We performed two repeated measures ANOVA, one for categorization performance in the two stimulus conditions and one for the detection performance in the one stimulus condition. The repeated measures ANOVA in the two stimulus condition revealed no main effects of hemifield ( $F(1,17) = 3.4$ ,  $p = 0.081$ ), TMS ( $F(1,17) = 0.71$ ,  $p = 0.41$ ) or contrast ( $F(2,34) = 1.99$ ,  $p = 0.153$ ). Most importantly we did not find an interaction effect between factors TMS and hemifield ( $F(1,17) = 1.76$ ,  $p = 0.2$ ), TMS and contrast ( $F(2,34) = 0.6$ ,  $p = 0.554$ ) or TMS, hemifield and contrast ( $F(2,34) = 0.15$ ,  $p = 0.86$ ) supporting the claim that TMS does not lead to phase-independent changes in excitability that could influence orientation categorization. Similarly, we found no main effect or interaction of TMS and/or hemifield on detection performance in the single stimulus condition (TMS:  $F(1,17) = 0.28$ ,  $p = 0.59$ ; hemifield:  $F(1,17) = 1.92$ ,  $p = 0.184$ ; TMS\*hemifield:  $F(1,17) = 0.33$ ,  $p = 0.573$ ). Our results speak against a significant effect of contrast asymmetries (first stimulus with low contrast versus second stimulus with low contrast) on TOJ performance and verify that participants utilized both stimuli equally to perform temporal order judgements.

We interpret the above presented findings as evidence that TMS did not have a general, time-unspecific effect on stimulus detection or orientation categorization, which could have indirectly led to a modulation of TOJ performance, but rather modulated perceived relative timing of the stimuli. Similarly we can conclude that severe asymmetries in stimulus contrast, potentially causing difficulties in orientation detection, do not lead to differences in TOJ performance and cannot cause modulations in performance.

## 4. Discussion

We tested the influence of  $\alpha$ -TMS on subsequent temporal order judgements at varying SOA's. 5 Pulses at 10 Hz were administered over left or right occipital cortex.  $\alpha$ -TMS was intended to entrain  $\alpha$ -oscillations in a local neural population. We probed temporal order perception of

two Gabor patches, at the spatial location presumably affected by the TMS entrainment, at 9 SOA's between 25 ms and 158 ms after the last TMS-pulse. Behavioral performance at every SOA was averaged to obtain a 133 ms long TOJ time-series. The frequency analysis of the TOJ time-series contralateral to TMS revealed a strong oscillation at our entrainment frequency of 10 Hz. We found no evidence of potentially confounding effects of excitability fluctuations on TOJ in our control conditions. In line with previous accounts of discrete perception we hypothesize that the rhythmic modulation in TOJ was caused by a TMS-evoked entrainment of occipital  $\alpha$ -oscillations. The phase of  $\alpha$ -oscillations has previously been related to so-called "perceptual windows" that serve to discretize visual input into compressed packages. Here we specifically tested the hypothesis that this compression leads to a deterioration of temporal order information. Depending on the relative timing to the last TMS pulse (SOA) the two stimuli fall either in the same or separate perceptual windows, leading to decreased or enhanced TOJ performance respectively. Our findings are in line with a "hard" theory of discrete perception suggesting that temporal order information is limited within perceptual windows. We provide causal evidence that suggests an involvement of occipitally entrained  $\alpha$ -oscillations in this process.

Correlational evidence for an involvement of the occipital  $\alpha$ -rhythm in the discretization of visual input is frequent (Busch et al., 2009; Haegens et al., 2011; Lőrincz et al., 2009; Milton and Pleydell-Pearce, 2016; Samaha and Postle, 2015; Valera et al., 1981; VanRullen, 2016; VanRullen and Koch, 2003; Vijayan and Kopell, 2012). Yet it was not clear if the  $\alpha$ -rhythm merely modulates excitability, leading to continuous fluctuations in visual performance or if it plays a causal role in the implementation of discrete non-overlapping perceptual windows. It is important to note that fluctuations in performance do not need to be all-or-none to support the "hard" version of temporal perception. All-or-none effects can rarely be expected due to noise in the phase entrainment and phase measurement methods or due to inter-individual differences in oscillatory frequency and optimal phase. We argue that it is sufficient to show that temporal perception itself fluctuates and that these fluctuations cannot simply be explained by changes in excitability (e.g. stimulus detection), leaving discrete temporal windows as the most plausible explanation.

Recent work shows that the  $\alpha$ -rhythm can be causally modulated via visual entrainment, leading to fluctuations in temporal parsing performance (Ronconi et al., 2018; Chota and VanRullen, 2019). While these studies help to link  $\alpha$ -oscillations and perception, they are limited since a visual entrainer passes various processing stages e.g. the LGN before arriving at V1. The LGN is hypothesized to project not only to V1, but also directly to higher cortical areas like V2 and V3 (Schmid et al., 2010). Strictly speaking every possible target of visual entrainment could serve as a potential source for the behavioral observations previously reported (Mathewson et al., 2010; Ronconi et al., 2018; Spaak et al., 2014; Chota and VanRullen, 2019). TMS allows for a direct and relatively localized interaction with endogenous cortical rhythms and is especially efficient at 10 Hz, even when individual  $\alpha$ -peak frequencies differ (Romei et al., 2010; Thut et al., 2011). Furthermore non-invasive TMS entrainment procedures have been successful in modulating behavior (Romei et al., 2010). As the target of our entrainment is confined to the early occipital cortex we can say with relative certainty that the occipital  $\alpha$ -rhythm gives rise to the perceptual effects demonstrated in this study. We therefore provide evidence for a causal link between occipitally entrained  $\alpha$ -oscillations and temporal order judgements.

Former experiments have mostly used integration versus segregation tasks (IvS), i.e. the two-flash fusion paradigm, to quantify temporal parsing performance (Ronconi et al., 2018; Samaha and Postle, 2015; Valera et al., 1981). These tasks present two stimuli in quick succession and subsequently probe participants' temporal segregation abilities (simultaneous vs. non-simultaneous, one vs. two). However, perceptually it is principally sufficient to detect changes in luminance over time, irrespective of the stimulus characteristics. Flicker fusion experiments show

that temporal changes can be detected at far higher frequencies than 10 Hz [Simonson and Brozek \(1952\)](#). TOJ's however require precise perception of temporal relationships between individual stimuli and cannot be solved purely by identifying transient changes in luminance. We therefore suggest that TOJ is better suited to investigate the effect of  $\alpha$ -phase on temporal perception.

Another complicating factor of IvS tasks is that the oscillatory phase facilitates or inhibits stimulus detection, potentially biasing responses by hiding stimuli from perception ([Busch et al., 2009](#); [Fellinger et al., 2011](#)). This raises the possibility that effects of phase on IvS performance are caused by inhibitory effects on single stimuli rather than time distortion effects caused by discrete sampling. One could also forward this objection against our paradigm. We therefore implemented several control conditions and analysis into our task. It is important to note that generally TOJ tasks should be relatively robust to the effects of visibility since relative timing judgements can be made with a clear percept of either stimulus, given that one stimulus reliably predicts the other as was the case here. Further, if certain phases of alpha would reduce stimulus visibility then we would expect this to influence TOJ performance at a frequency of 20 Hz instead of 10 Hz. As described in [Section 3.2.3](#) this is because alpha phase would affect both stimuli sequentially within a 100 ms cycle. We also hypothesized that if our findings were the result of phase-dependent  $\alpha$ -inhibition, this should lead to a general reduction in the visibility of the target stimulus and reduce performance especially for low contrast conditions. Furthermore, such an effect would likely result in a periodic modulation of single-stimulus reports. Our control conditions however demonstrate that performance was comparable between contrast conditions and single-stimulus responses were not modulated in the 5 to 15 Hz range, indicating that temporal order judgments could be performed under conditions of reduced visibility and were unbiased by excitability fluctuations. In addition we removed all single-stimulus reports from the main TOJ-analysis. From our control results we conclude that modulations of stimulus visibility were not the main determinant of TOJ performance fluctuations.

While the effect of  $\alpha$ -TMS on the contralateral visual field was somewhat expected, we were surprised to find a TMS-evoked oscillatory pattern also in the ipsilateral visual field. The ipsiTMS time-series showed a relatively weaker amplitude, oscillated at around 8 Hz and fluctuated in antiphase compared to its contralateral counterpart for at least 160 ms. It is unlikely that the magnetic field of the TMS-pulse directly interacted with the contralateral hemisphere because first, all subjects reported phosphenes only contralaterally, and second, TMS pulses seemed to have opposing effects depending on the stimulus location. Previous work has shown that  $\alpha$ -TMS can affect target detection performance in the visual field contra- and ipsilateral to the entrainment site, possibly through a transcallosal “push-pull” effect ([Romei et al., 2010](#)). Our findings suggest that  $\alpha$ -phase is modulated by this network effect, potentially releasing one hemisphere from inhibition when the other hemisphere enters a state of inhibition. One possible interpretation of the frequency difference is that the ipsilateral oscillation reflects a slower attentional rhythm that is usually placed in the 3 to 8 Hz range, potentially lower than our frequency analysis allows us to investigate ([Fiebelkorn et al., 2018](#); [Fiebelkorn and Kastner, 2019](#); [VanRullen, 2013](#); [VanRullen et al., 2007](#)). Another possibility is that the change in frequency relates to differences in  $\alpha$ -power between hemispheres. As mentioned before a push-pull effect might lead to a significant reduction in 10 Hz power in the ipsilateral hemisphere ([Romei et al., 2010](#)). Since this oscillatory frequency is likely relevant for stimulus processing the brain might hypothetically try to compensate by modulating excitability at a lower frequency.

Some TMS studies include additional control manipulations, e.g., a sham condition mimicking the auditory clicks of the TMS coil, or an arrhythmic condition with stimulation patterns of equal duration and pulse number but irregular intervals. Sham controls aim to test if neural entrainment could be caused by the rhythmic auditory stimulation instead of the magnetic field of the TMS coil. Arrhythmic stimulation al-

lows experimenters to determine whether rhythmicity is necessary, or whether any pulse train (or potentially only the last pulse) could suffice to give rise to the observed modulations in behavior. We do not believe that our experimental task is concerned by these confounds, for the following reasons. First, the auditory signature of TMS pulses affects both hemispheres at the same time; yet we observed an anti-phasic pattern in the contra- versus ipsilateral TOJ time-series. Second, concerning the potential behavioral effects of single pulses (or arrhythmic trains), while we cannot exclude that these may have led to comparable effects (alpha-band modulation of TOJ time courses), we do not believe that this would have changed our primary conclusion (causal involvement of alpha oscillations in TOJ tasks). In accordance with current models of neural entrainment, it is likely that single TMS pulses could still lead to a weak phase reset of alpha oscillations, which in turn would modulate behavior. Importantly the frequency of our behavioral fluctuations shows a clear periodicity in the alpha range. Irrespective of which aspect of the stimulation pattern led to this effect (i.e., the stimulation frequency, the number of pulses or the very last pulse), this clearly speaks to an involvement of entrained oscillations in temporal order judgments. Rhythmic pulses as opposed to single pulses merely increase our ability to observe these oscillations behaviorally. Another popular control condition involves stimulating at the flanker frequencies of the frequency of interest (e.g. 6 and 14 Hz). These controls aim to show that the entrainment effect is confined to a specific frequency. The lack of such a control is certainly a minor limitation of our study, however we believe that previous studies including these controls have convincingly shown that neural entrainment has highly frequency specific effects at the alpha rhythm (e.g. [Gulbinaite et al., 2017](#); [Herrmann, 2001](#); [Romei et al., 2010](#); [Wiesman and Wilson, 2019](#)). Future studies will have to verify if the behavioral effects found in our study are indeed correlating with changes in oscillatory activity using EEG.

The phase of  $\alpha$ -oscillations is predictive of cortical excitability ([Busch et al., 2009](#); [Dugué et al., 2011](#)), of neuronal firing rates ([Haegens et al., 2011](#); [Lórinicz et al., 2009](#); [Vijayan and Kopell, 2012](#)) and of the amplitude of gamma oscillations ([Osipova et al., 2008](#); [Voytek et al., 2010](#)). As these neural signatures have been implicated in neuronal processing it seems logical that visual processing is concentrated on specific reoccurring intervals. The brain might use these naturally occurring periodicities, in the form of oscillations, to reduce the complexity of incoming information by compressing it into discrete packages. Our findings suggest that this compression results in the loss of temporal relationship between two stimuli. Note that the visual system is very robust to subsampling in the 100 ms range ([VanRullen, Zoefel, & Ilhan, 2014](#)), presumably because visual information is highly redundant, allowing the visual system to reduce complexity via periodic discretization without losing too much relevant information.

In this study we successfully demonstrated that TMS entrainment at 10 Hz leads to a causal rhythmic modulation of temporal order judgements. This modulation was evident in the majority of subjects, shown by a strong inter-individual phase coherence in individual TOJ time-series. Furthermore we found that ipsilateral TOJ time-series were modulated in antiphase to their contralateral counterparts; this cross-hemispheric effect is unlikely to be caused directly by the TMS, but may result from a secondary trans-callosal pathway. We hypothesize that the visual system periodically discards temporal order information in order to reduce the complexity of incoming visual information. Further, we hypothesize a potential causal involvement of occipitally entrained  $\alpha$ -oscillations in this discrete sampling mechanism .

#### Author statement

**Samson Chota:** Conceptualization, Methodology, Software, Formal Analysis, Investigation, Writing - Original Draft **Phillipe Marque:** Resources **Rufin VanRullen:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition

## Data availability

The raw behavioral data will be made publicly available at the time of publication. Individual scripts can be acquired by contacting the corresponding author.

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