Cortical stimulation consolidates and reactivates visual experience: neural plasticity from magnetic entrainment of visual activity

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Delivering transcranial magnetic stimulation (TMS) shortly after the end of a visual stimulus can cause a TMS-induced ‘replay’ or ‘visual echo’ of the visual percept. In the current study, we find an entrainment effect that after repeated elicitations of TMS-induced replay with the same visual stimulus, the replay can be induced by TMS alone, without the need for the physical visual stimulus. In Experiment 1, we used a subjective rating task to examine the phenomenal aspects of TMS-entrained replays. In Experiment 2, we used an objective masking paradigm to quantitatively validate the phenomenon and to examine the involvement of low-level mechanisms. Results showed that the TMS-entrained replay was not only phenomenally experienced (Exp.1), but also able to hamper letter identification (Exp.2). The findings have implications in several directions: (1) the visual cortical representation and iconic memory, (2) experience-based plasticity in the visual cortex, and (3) their relationship to visual awareness.

TMS-induced phosphene flashes are generally perceived to be colorless or palely colored flashes of light, shaped as blobs, radial wedges, or quadrantic fills. However, the visual experiences induced by TMS can differ when visual cortex is already at a non-baseline state at the time of stimulation. For example, if the participant has been pre-adapted using colored light, then TMS elicits a phosphene that is tinted with the adapted color3,4. This visual percept reflects the altered activity and excitability states across neurons in the adapted cortex. It is hypothesized that the subset of neurons stimulated by the adapting color becomes more easily activated by TMS following the adaptation period3,4.

In the course of conducting our research into TMS-induced replay, it was occasionally noted that TMS alone would sometimes elicit a replay-like effect. The participant would see features of visual stimuli from preceding trials, even though the TMS delay to the visual stimulus (6–10 seconds) were much longer than the usual effective periods which are within 400 ms. These events tended to occur after extended testing of the replay effect, especially if the same visual stimulus was used repeatedly. This suggested that organized cortical excitability...
states were persisting longer than usual, as if the cortical states were becoming entrained due to repeated pairings of TMS with a visual stimulus.

In the current study, we examine this entrainment phenomenon directly. Experiment 1 characterizes the subjective experience of an entrained TMS replay based on subjective strength ratings, and examines the conditions necessary to induce entrainment. Experiment 2 validates the phenomenon by measuring performance levels on an objective target discrimination task, where the TMS-entrained replay operated as a masker to suppress visibility of a target. This functional measure of the effect also tests the hypothesis about the underlying neural mechanism that the replay and ordinary percepts share early visual cortical circuits.

The basic structure of the experiments included two types of trials. In learning trials, a visual stimulus is followed by TMS, causing a replay effect each time. These trials are repeated back-to-back in such a paired fashion of visual stimulus and TMS in order to induce an entraining effect. In subsequent test trials, TMS is delivered without a visual stimulus. While TMS in isolation normally results only in a phosphene, some participants would see a replay of the visual stimulus used during these test trials, which we define as a “TMS entrainment effect”.

Results

Experiment 1: timecourse of subjective experience, and conditions necessary for entrainment. To examine the subjective experiences of the TMS entrainment effect, we asked the participants to rate the vividness of replay percepts throughout the learning and test phases. This produced ratings of standard replay percepts and the entrained vividness of replay percepts. The TMS entrainment effect, we asked the participants to rate the necessary for entrainment

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ing phosphenes in response to TMS. Of those 17, 14 reported a replay percept. Finally, among the 14 participants who perceived re-plays, 10 reported perceiving entrainment. That is, in the test phase of the experiment, they continued to report seeing a replayed visual stimu-

lus in response to TMS (i.e., the rating score of the test phase was higher than zero, t(9) = 4.09, P = .003, two-tailed). A timecourse of their reports and the average scores across participants, are shown in Figure 2 (in red). A replotting of the figure to include all the participants who perceived re-plays regardless whether or not perceiving entrainment (n = 14) in the average, compared to the participants who perceived entrainment (n = 10) is shown in Supplementary Figure 1. It shows, that for all the participants who reported a replay percept, the average result still indicated the entrainment effect [11th–15th trials’ mean±SEM = 1.64 ± 0.49; the Student’s one-sample t-test showed that the mean was significant higher than 0, t(13) = 3.44, P = .004, two-tailed].

To examine the conditions necessary for inducing entrainment, we ran a control version of the learning phase (Fig. 1b) in which the visual stimuli and TMS were presented out of phase, separated by 3 seconds. This counter-phase presentation exposed the participant to the same number of TMS pulses and visual stimuli, but did not allow the two types of stimuli to interact to cause a replay. It tested the subjective strength and its timecourse of the entrainment effect following the learning phase.

An experimental run consisted of 15 trials (illustrated in Fig. 1a). The first ten were replay learning trials, where a visual stimulus was followed by TMS with a 300 ms stimulus onset asynchrony (SOA). The last five trials were test trials, containing only TMS. Inter-trial intervals were 6 seconds, to allow the TMS units to recharge. Following each trial, the participant gave integer ratings ranging from 1–9 for the vividness of TMS-induced replay percepts. A rating of 9 would mean that TMS caused them to see a vivid duplication of the visual stimulus. When they perceived a TMS-induced phosphene but not the replay, they were instructed to give the rating number as 0.

Because the visual effect of TMS varies across participants (some participants see no effects at all in response to TMS, not even a phosphene), we performed a preliminary screening to determine the proportion of participants who saw phosphenes, replay and entrainment (see Methods for details). Among 19 participants recruited in the preliminary screening, 17 participants reported seeing phosphenes in response to TMS. Of those 17, 14 reported a replay percept. Finally, among the 14 participants who perceived re-plays, 10 reported perceiving entrainment. That is, in the test phase of the experiment, they continued to report seeing a replayed visual stimu-

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possibility that entrainment came simply as an accumulated effect of the stimulus components, rather than the repeated experience of joint stimulations. Results of this experiment are shown in Figure 2, in blue.

Ratings during the test periods following replay learning trials were significantly higher than those following control learning trials. A two-way repeated-measures ANOVA (n = 10) with experimental condition (entrainment, control) and trial number (11–15) as within-subject factors showed a main effect for condition \( F(1,9) = 11.91, P = .007 \), with ratings much higher in the entrainment condition [mean ± SEM = 2.28 ± 0.56] than in the control condition [0.22 ± 0.10]. The vast majority of control trials, including learning and test trials, elicited ratings of zero. This indicated that the repeated replay interaction between paired TMS and visual stimuli is the necessary condition to cause the entrainment.

Phenomenologically, most participants described the TMS-entrained replay to be similar to the TMS-induced replay, but less vivid. This was reflected in lower rating scores for the 1st trial of the test phase [11th trial mean = 2.90 ± 0.61], as compared to the last trial of the learning phase [10th trial mean = 5.98 ± 0.62; t(9) = 3.49, \( P = .007 \); the Student’s t-Test, two-tailed, paired comparison].

**Experiment 2: functional characterization—validation and mechanisms.** Having characterized the entrained TMS-induced replay at a subjective and phenomenological level, in Experiment 2 we characterized the effect at a functional level. This served to objectively validate the effect in a task robust to criterion shifts and other cognitive judgment biases. It also addressed the behavioral and physiological sides of the effect. In particular, we examined the question as to whether the TMS-entrained replay shares similar neural mechanisms with the processing of physical visual stimuli.

To obtain objective verification of the entrainment effect, we measured changes in performance on a target discrimination task (Fig. 3). We presented a masking stimulus on the screen, followed by one of eight target letters. The mask and target were separated by 300 ms, too long of an SOA for there to be a direct forward masking effect. However, we used TMS to replay the mask just prior to the target, at a timing where a visual mask normally creates effective forward masking.

If the perceptual replay and entrainment effects reflect visual cortex activity which is similar to that found in normal visual processing, then we would expect the replayed mask to function similarly to an optimally timed “presentation” of a physical mask. This would result in reduced performance on the letter identification task. Thus, reduced performance with TMS-induced and TMS-entrained replay would provide objective evidence for the validity of the subjective phenomenology.

Participants (n = 13) ran four blocks of trials, diagrammed in Figure 3a. Each block began and ended with a series of no-TMS baseline trials, in which only the physical mask and target were presented. In the middle of each block, TMS-replay learning trials and TMS-test trials were intermixed. In addition to using these three trial types within each block, we also varied the mask and target positions across blocks so that they were aligned in half the blocks and misaligned in the other half (Fig. 3b).

Since masking requires alignment both in time and space, specific evidence for a low level visual masking effect mediated by TMS replay and entrainment could be found using two kinds of conditional comparisons. Spatially aligned masks should result in a larger masking effect, i.e. lower performance than misaligned masks, but only in the TMS-replay learning and TMS-test trials, where TMS was used to replay the mask to the visual system at the proper timing; meanwhile in the baseline no-TMS trials, it should make no difference whether masks were aligned or misaligned. In a complementary test, the presence of TMS should result in lower performance than in no-TMS trials, but specifically when there was an aligned mask to replay; when masks and targets were misaligned, it should not matter whether TMS is present or not.

Results are shown in Figure 4. Target identification accuracy was subjected to a two-way repeated-measures ANOVA (n = 13) with the target-mask alignment (aligned, misaligned) and TMS conditions as within-subject factors and block as a between-subject factor. Statistical analysis of target identification accuracy showed a significant main effect for target-mask alignment \( F(1,13) = 10.72, P = .007 \), with ratings much higher in the entrainment condition [mean ± SEM = 2.90 ± 0.56] than in the control condition [0.22 ± 0.10]. The vast majority of control trials, including learning and test trials, elicited ratings of zero. This indicated that the repeated replay interaction between paired TMS and visual stimuli is the necessary condition to cause the entrainment.

**Figure 3** | Stimuli in Experiment 2. Participants’ performance in a letter discrimination task was tracked across learning, test, and baseline trials. (a) Timelines of the trial types and block structure. In learning trials, mask preceded the target by 300 ms, and TMS preceded the target by 50 ms. In test trials, no mask was presented. Trials included only TMS and target. In baseline no-TMS trials, no TMS pulse was delivered. Trials included only mask and target. (b) Illustration of the eight possible targets, the mask, and the two possible locations where the target and mask could appear.

**Figure 4** | Results of Experiment 2. Mean accuracy of target letter discrimination as a function of mask conditions. Asterisks indicate statistically significant differences between the conditions \( (P < .05) \).
conditions (no-TMS, TMS-replay learning, and TMS-test) as within-subject factors. We found both main effects of target-mask alignment \([F(1,12) = 5.52, P = .04]\) and TMS conditions \([F(2,24) = 4.74, P = .02]\). Further, the two-way interaction was also significant \([F(2,24) = 4.60, P = .02]\).

Subdividing the main effect of target-mask alignment (Figure 4, white bars vs. black bars), we found it was conditioned upon the presence of TMS. That is, aligned masks led to lower performance than misaligned masks, but only when TMS replayed the mask just prior to the target. One-way ANOVA comparing aligned and misaligned masks showed significant differences in the TMS-replay learning trials \([F(1,36) = 8.75, P = .01]\) and the TMS-test trials \([F(1,36) = 5.20, P = .03]\), but not in the baseline no-TMS trials, \([F(1,36) = .88, P = .36]\). The latter result indicates that the physical mask alone was indeed ineffective at the 300 ms SOA, since performance did not vary depending on target-mask alignment. Thus, a spatially-specific masking effect arose only in the TMS-replay learning and TMS-test trials. When TMS replayed a spatially aligned mask, it resulted in lower target discrimination performance than when TMS replayed a spatially misaligned mask. In this, the replayed mask had functional effects similar to a physical mask, and this applied to both TMS-replay learning and test trials.

Subdividing the orthogonal main effect of TMS condition, we found that the TMS impairment on target identification was conditioned upon the presentation of aligned masks. TMS-replay learning and TMS-test trials resulted in lower performance only when the target was aligned with the mask \([F(2,48) = 8.38, P = .001]\), pairwise differences (Tukey’s test with alpha level = .01, two-tailed) shown among white bars in Fig. 4. When the target and mask were misaligned, TMS did not hamper target identification \([F(2,48) = 1.04, P = .36]\, black bars in Fig. 4). Thus general forms of TMS interference, such as masking from the TMS phosphene cannot account for the pattern of results. If the impairment of performance was caused by the phosphene per se, we would expect similar decrement of target identification whether or not the target was presented aligned with the mask. Further, it has previously been found that TMS caused almost no change in contrast detection thresholds when TMS preceded the target by 50 ms\(^{11}\). Thus, a direct masking effect of TMS cannot explain the systematic pattern of masking we found here.

Finally, to see if the performance dropped could be attributed to a general fatigue effect, we compared the baseline no-TMS trials at the beginning of each block with the no-TMS trials conducted at the end of each block. Results showed no difference in target identification accuracy, whether comparing on the basis of trials where target and mask were aligned \([75.8\% \text{ vs. } 73.1\%, t(12) = .77, P = .46]\) or misaligned \([70.1\% \text{ vs. } 69.2\%, t(12) = .19, P = .85]\; the Student’s t-Test, two-tailed, paired comparison). Since performance was stable across the periods immediately before and after the TMS trials, it is unlikely that the effect in the intervening TMS trials could be accounted for by fatigue or any other cumulative effects.

**Discussion**

In two experiments, we characterized an entraining effect found after the repeated pairing of TMS and a visual stimulus. Each pairing event, at a stimulus-onset-asynchrony of 300 ms (Experiment 1) or 250 ms (Experiment 2), was designed to produce a perceptual replay interaction. The repeated interactions between TMS and a visual stimulus led to an entrainment of the interaction effect, such that the physical visual stimulus was no longer necessary to elicit the effect. While the perceptual replay interaction normally requires pairing of visual stimuli with TMS within a few hundred milliseconds, entrained participants saw replayed stimuli in trials containing only TMS, which occurred 6–30 seconds after the last trial containing the visual stimulus.

Participants reported their experiences of the entrained effect as being phenomenally similar, but weaker in strength, compared to the direct replay effect. We found that the emergence of the entrainment effect required the repeated close pairing of TMS and visual stimuli; delivering the same number of stimuli out of phase with the TMS did not lead to any entrainment.

When participants were given a target discrimination task, their performance could be disrupted by both replayed and entrained masks. The replayed and entrained masks needed to be aligned with the target to be effective. Thus, the masking effect of the replayed and entrained stimuli showed the same spatial dependency found with physically presented masks. This provides objective validation of the subjective reports, and also supports the hypothesis that replayed and entrained visual percepts are processed by mechanisms similar to those of regular visual processing.

A previous study of the perceptual replay or ‘visual echo’ examined the effect of the visual surround on replayed images, measuring the amount of tilt repulsion between the two. Results showed that the replayed image interacted with the visual surround presented at the time of replay, not at the time of the original visual stimulus presentation. This suggests that the replay effect involved a recapitulation of normal low-level visual processing. Our results from the TMS-replay learning trials provide further support for this hypothesis, as masking interactions are also mediated in early visual cortex. Further, the results from the TMS-test trials extend the same implications to the entrained percepts.

One may alternatively consider the entrained percepts as visual imagery extracted by TMS\(^{11,12}\); however, the temporal and spatial specificity found in our results suggest a representation more closely locked to the original ‘real’ retinally-driven percept. Our data, particularly the spatial selectivity, should be interpreted in the context of the continuum from percept to iconic storage to visual memory.

Broadly speaking, the structure of the experimental design resembles classical conditioning. Considering the TMS replay as the conditioned response (CR), the unconditioned stimulus (UCS) which induces reflexive neural response of the TMS replay (i.e., visual stimulus followed by TMS) always accompanies with TMS; it could be regarded as a form of the classical conditioning. After the repeated experience of the TMS replay induced by the paired association, TMS alone (conditioned stimulus, CS) is able to induce the CR, i.e., TMS replay. During the learning, it is critically necessary that the UCS and CS have to appear closely in time (in fact, they are overlapping in our associative learning procedure), similar to the classical conditioning procedures. However, there is one notable dissimilarity. In the case of classical conditioning, the CS does not have a selective causal power to induce the response at the outset. In the extreme case, a conditioning is possible with CS following the UCR in time. In our associative learning of TMS-entrained replay, the CS (i.e., TMS) can be alternatively considered as part of the UCS (because the visual stimulus has to be followed by the TMS) and thus have the selective causal power to induce a visual percept even before the learning. Presumably, TMS elicits stronger interference with the neural system than ordinary sensory input that may make the learning more efficient to alter the perception. Indeed, 10 trials is a small number of repetitions, relative to the classical conditioning procedure.

Our finding of the TMS-entrained replay should be added to the short list of phenomena in which conscious visual experience is triggered without direct retinal input, along with phenomena such as visual afterimage and synesthesia. As with afterimages, the TMS-entrained replay involves adaptive neural processes in response to TMS-induced replay. However, the color and brightness of the replay percept is not reversed as in a negative afterimage, but rather a graded version the original visual stimulus. Synesthesia results from an intrinsic association between different sensory modalities, presumably owning to local cross-activation\(^{13,14}\) or long-range disinhibited hierarchical feedback\(^{15}\). The TMS-entrained replay, by contrast, is formed through an associative learning between visual input and direct visual cortex stimulation. Further exploration on the
interaction among different sensory inputs and direct brain stimulation is required to understand how the experience-based neural plasticity, within or across modalities, contributes to perceptual awareness.

Based on previous cortical registration data, the optimization of phosphene perception likely results in a coil location near the V2/V3 border, but the nature of neurophysiological effect elicited by TMS at its site(s) of action is unclear. Single-cell recordings from cat striate cortex during joint stimulation with TMS and visual input have revealed both inhibitory and facilitatory effects on visual activity. When paired-pulse TMS was tested, the result was a magnification of whatever single-pulse effect had been found in the particular cell. The effect was independent of ISI (inter-stimulus-interval), but only ISIs ranged from 2 to 30 ms were tested. In humans, the effects of TMS to occipital cortex have been investigated by measuring the thresholds at which paired-pulse TMS elicits phosphene percepts. These studies find a facilitatory interaction between the paired pulses, which fairly stable up to ISIs of about 100 ms. Beyond 100 ms, whatever single-pulse effect had been found in the particular cell. The coil was held in position on the participant's occipital scalp using a chinrest that was clamped to the desk. Paired-pulse monophasic TMS with a 50 ms interval was generated by a Magstim BiStim System through the Magstim 70 mm figure-eight coil. According to our pilot investigation, paired-pulse TMS elicits stronger effect for TMS-induced relay (content is more vivid, relay is more frequently observed) than single-pulse TMS. Therefore, we arbitrarily set the screening criteria that participants had to perceive TMS-induced phosphene in response to the paired-pulse TMS at intensity levels below 70% of maximum stimulator output to allow the room to increase the TMS intensity for TMS-induced relay. The average TMS intensity was 79.3% and 77.7% in Experiment 1 and 2, respectively, and the TMS intensity used for each participant was listed in Supplementary Tables (1 and 2).

To the extent that phosphene mechanisms resemble the ones underlying perceptual replay, this suggests that the two pulses of TMS with a 50-ms ISI used in our experiment may have interacted in a functionally facilitatory. However, one major difference between our experiment and the other experiments lies in the state of visual cortex at the time of TMS. The previous experiments were conducted either with no visual stimulus, or with TMS slightly before a visual stimulus. In our case, however, TMS came well after the visual stimulus around 250–300 ms. The pre-existing cortical state at the time of stimulation is known to affect the elicited activity and functional outcome.

It is important to be aware that the functional facilitation between the paired-pulse TMS is a separate issue from the physiological inhibition or facilitation of neural activity. Thus it is unclear whether TMS in the current study triggers replay and consolidation via facilitation or inhibition of neural network activity. A facilitatory explanation would be that the replay results from a re-activation in the visual cortex that somehow recapitulates the original activity pattern evoked by the visual stimulus. An inhibitory explanation of replay could be based on the interruption of mechanisms that normally suppress long-lasting visual representations from reaching consciousness. Further study is required to explore the underlying mechanism. For example, assuming that the replay is a re-appearance of the visual stimulus (i.e., the facilitation hypothesis), the replay should be able to improve task performance if a target is replayed. If the replay is the release of already-present visual representations via disruption of suppressive networks (i.e., the inhibition hypothesis), then we might not expect a performance increase, since no additional information becomes available.

In conclusion, we found an entrainment effect that human visual cortex can be “rewired” by TMS within a short period (1–2 minutes) to alter the visual response to a fixed TMS input. The current finding of associative replay strongly suggests that a conscious visual percept is not mechanically triggered by the retinal or TMS input in a one-to-one fashion. Rather, it is a result of its interaction with the prior internal state of the visual cortex, which reflects past experiences and associative learning.

Methods

Participants. Twenty-two healthy adults who had no neuropathological history and passed the screening test for TMS-induced relay (see below) participated in the current study. Experiment 1 included 16 naive participants. Experiment 2 included 13 participants, 5 of whom also participated in Experiment 1 (WL, RD, SG, JY, and AM). The current study was approved by the Caltech Institutional Review Board, and all participants gave informed consent before the experiment.

Setup. Visual stimuli were presented in a 22 inch monitor (LaCie electron 22 blueIV, 1024 × 768 resolution), controlled by a Macintosh desktop with MATLAB Psychtoolbox software (Experiment 1) or by a PC desktop with the Vision Egg package for the Python programming environment (Experiment 2). Participants sat in front of the monitor at 34-cm viewing distance in a dark room, stabilized by a chinrest that was clamped to the desk. Paired-pulse monophasic TMS with a 50 ms interval was generated by a Magstim BiStim System through the Magstim 70 mm figure-eight coil. According to our pilot investigation, paired-pulse TMS elicits stronger effect for TMS-induced relay (content is more vivid, relay is more frequently observed) than single-pulse TMS. The coil was held in position on the participant’s occipital scalp using a modified locking arm and clamp, which was mounted onto the same frame as the chinrest. The Magstim Bistim System was controlled by the same computer and software as the visual stimulus presentation, via a Measurement Computing 1208 FS USB DAQ module.

Participant screening and TMS optimization. We screened participants to identify those who found the stimulation agreeable. According to our pilot investigation, the required TMS intensity for TMS-induced relay was larger than phosphene. Therefore, we arbitrarily set the screening criteria that participants had to perceive TMS-induced phosphene in response to the paired-pulse TMS at intensity levels below 70% of maximum stimulator output to allow the room to increase the TMS intensity for TMS-induced relay. The average TMS intensity was 79.3% and 77.7% in Experiment 1 and 2, respectively, and the TMS intensity used for each participant was listed in Supplementary Tables (1 and 2). Coil position was optimized to maximize the perceptual intensity of the TMS-induced phosphene in the lower-right visual field of each participant (see below for more details).

We explored how TMS works. Participants were given a 40%-strength paired-pulse stimulation on the inside of the forehead to feel the muscle twitch, and told that a similar or somewhat stronger muscle twitch may be felt on the scalp and/or shoulder during the TMS. The figure-eight coil was then placed on the scalp 2 cm to the left of and 2 cm above the inion. The TMS intensity started at 40% and increased in 5% steps. Participants were requested to report how they felt and what they saw during the TMS pulses. If they spontaneously reported seeing a brief flash of light, we explained that the flash/light was caused by TMS and called a phosphene. We then adjusted the TMS coil location and/or tilt angle, and requested the participants to describe the location and area of the phosphene while maintaining their fixation. The coil was adjusted to maximize the phosphene’s coverage of the lower-right visual field, particularly where the visual stimulus was presented. We then fixed the TMS coil location with a coil clamp.

The TMS-induced relay screening test was further conducted on the participants who passed the phosphene screening procedure described above. A black 5 pixels (5 × 5 × 5 mm), luminance of 4 cd/m2 was presented at the center as the fixation point against a gray background (luminance 4.80 cd/m2). A visual stimulus was presented 300 ms prior to the TMS in the lower-right quadrant of the visual field overlapping with the phosphene, since the relay of the visual features were often seen as embedded within a broader phosphene. Different types of visual stimuli were tested for each participant due to the individual variation. Some participants mainly reported the contour of the visual stimulus, some mainly reported the color, and some reported both. The optimal visual stimulus for relay for each participant was determined, choosing from two geometric shapes (an oblique line and a disk) and four colors (red, green, black, and white). The oblique line (283 pixels length, 20 pixels width) was presented from the fixation point (0 × 0) to the lower-right quadrant. The disk was centered in the fixation point with 200 pixels in radius. Participants were given several trials of the relay until their perceptual reports stabilized and they felt able to give a principled vividness rating.

In Experiment 1, 19 participants were recruited in the screening test. Of those 19, 17 participants reported seeing phosphenes in response to TMS, thus only 2 (11%) of participants failed to see the phosphene at all. Various studies of phosphene stimulation threshold have found that approximately 40% of participants do not experience phosphenes. The lower rate of failure (i.e. the higher rate of success) found in the current study could stem from the experimental parameters, especially in our use of paired-pulse TMS with a 50 ms ISI, in contrast to other studies which used single-pulse or paired-pulse with shorter ISI. Additionally, initial screening procedure could be also a factor. Whereas initial screening for phosphenes is typically tested in total darkness, Kammer et al. and we both tested participants in dimly lit environment while facing a uniformly lit background on the monitor. Often, participants’ first percepts are described as a shuddering of some portion of the visual scene, and then once they direct their attention to that phenomenon, they see it as flashes of brightness. Perhaps this initial shudder effect is not visible in the dark, and thus some participants cannot get that first toehold toward the full effect.

Among the 17 participants who saw phosphene, 14 reported a replay percept for one or more visual stimuli. For each of these participants, the visual stimulus that was most strongly replayed by TMS was chosen as the stimulus for the main experiments (listed in Supplementary Table 1). In Experiment 2, all the participants were recruited from our database who passed screening test for TMS-induced relay.

Experimental design. Experiment 1. Stimuli were the same as in the relay screening procedure. Each run consisted of two phases: learning phase (10 trials) and test phase (5 trials). In the learning phase, each trial consisted of a visual stimulus presented for 100 ms followed by paired-pulse TMS (inter-pulse interval 50 ms). In the test phase, only the paired-pulse TMS was delivered, no visual stimulus. Inter-trial interval was

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6 secs, allowing TMS system to recharge. Entrainment and control conditions differed only in the learning phase, where stimulus onset asynchrony between the visual stimulus and TMS was either 300 ms (entainment) or 3000 ms (control). Numbers of runs for each participant is listed in Supplementary Table 1. Participants rated the strength of replay in each trial on a discrete 0–9 scale by pressing a number on the keyboard.

**Experiment 2.** A white fixation cross (4 pixels × 4 pixels, luminance 99.7 cd/m²) was presented against a gray background (luminance 11.9 cd/m²). A pattern mask (30 pixels × 40 pixels) consisting of a superimposition of all eight possible target letters (A, H, N, E, O, V, T, X, line width of 2 pixels, line luminance 60.8 cd/m²) was used for TMS-induced replay. Target letters were the same size as the pattern mask, with brightness determined through a staircase procedure for each participant in a pretest. The pattern mask and the target letter were positioned at two possible locations in the lower-right quadrant, at 0° = −42° and −66°, both at 9.7° from the fixation point. Both locations were within the participants' reported phosphene area.

Target-mask alignment relationship (aligned, misaligned) and TMS conditions (no-TMS, TMS-replay learning, and TMS-test) were manipulated as the within-subject factors. The target location was at the same (aligned) or different (misaligned) locations as the pattern mask, run in different blocks with the order of blocks randomly assigned to each participant. Each block consisted of 40 trials with the following structure: 10 non-TMS trials, 10 entrainment trials, 5 alternations of one test trial followed by one entrainment trial, and 10 no-TMS trials. In the no-TMS (i.e., physical mask baseline) trials, the pattern mask was presented for 50 ms, followed by the target letter presented for 10 ms, with a stimulus onset asynchrony of 300 ms. The TMS-replayed learning condition (i.e., TMS-induced replay) was the same as the no-TMS condition, with the addition of paired-pulse TMS (with a 50-ms inter-pulse interval), which was delivered following the pattern mask with 250 ms delay in order to induce the replay. In the TMS-test condition (i.e., TMS-entrained mask), no physical pattern mask was presented. TMS and the letter target were presented at the same timing as the TMS-induced mask condition. In all conditions, participants were asked to identify the letter target from the eight possible letters, and to guess when they were unsure.

1. Walsh, V. & Pascual-Leone, A. Transcranial magnetic stimulation: A neurochronometrics of mind. The MIT Press, Cambridge, MA. (2005).
2. Meyer, B. U., Diehl, R., Steinmetz, H., Britton, T. C. & Renecke, R. Magnetic stimuli applied over motor and visual-cortex: Influence of coil position and field polarity on motor-responses, phosphenes, and eye-movements. *Electroencephalogr. Clin. Neurophysiol.* Suppl. 43, 121–134 (1991).
3. Silvanto, J., Muggleton, N. G., Cowey, A. & Walsh, V. Neural adaptation reveals state-dependent effects of transcranial magnetic stimulation. *Eur. J. Neurosci.* 25, 1874–1881 (2007).
4. Silvanto, J., Muggleton, N. G., Cowey, A. & Walsh, V. State-dependency in brain stimulation studies of perception and cognition. *Trends Cogn. Sci.* 12, 447–54 (2008).
5. Wu, D. A., Kamitani, Y., Maeda, F. & Shimojo, S. Interaction of TMS-induced phosphenes and visual stimuli. *J. Vis.* 1(3), 198 (2001).
6. Wu, D. A. & Shimojo, S. Transcranial magnetic stimulation (TMS) reveals the content of post-perceptual visual processing. *J. Vis.* 4(8), 47 (2004).
7. Neill, H., Wu, D. A. & Shimojo, S. TMS induces detail-rich “instant replays” of natural images. *J. Vis.* 7(9), 276 (2007).
8. Wu, D. A., Neill, H., Hoef, F. & Shimojo, S. TMS “instant replay” validated using novel double-blind stimulation technique. *J. Vis.* 7(9), 275 (2007).
9. Jolij, J. & Lamme, V. A. F. Transcranial magnetic stimulation-induced ‘visual echoes’ are generated in early visual cortex. *Neurosci. Lett.* 484, 178–181 (2010).
10. Kammer, T., Puls, K., Strasburger, H., Hill, N. J. & Wichmann, F. A. Transcranial magnetic stimulation in the visual system. I. The psychophysics of visual suppression. *Exp. Brain Res.* 160, 118–128 (2005).
11. Kosslyn, S. M. et al. The Role of Area 17 in Visual Imagery: Convergent Evidence from PET and rTMS. *Science* 284, 167–170 (1999).
12. Silvanto, J. & Cattaneo, Z. Transcranial magnetic stimulation reveals the content of visual short-term memory in the visual cortex. *Neurommage* 50, 1683–1689 (2010).
13. Ramachandran, V. S. & Hubbard, E. M. Synaesthesia: A window into perception, thought and language. *J. Conscious. Stud.* 8, 3–34 (2001).
14. Hubbard, E. M. & Ramachandran, V. S. Neurocognitive mechanisms of synaesthesia. *Neuron* 48, 509–520 (2005).
15. Grossenbacher, P. G. & Christopher, T. L. Mechanisms of synesthesia: Cognitive and physiological constraints. *Trends Cogn. Sci.* 5, 36–41 (2001).
16. Kammer, T., Puls, K., Erb, M. & Grodd, W. Transcranial magnetic stimulation in the visual system. II. Characterization of induced phosphenes and scotomas. *Exp. Brain Res.* 160, 129–140 (2005).
17. MolaZde, V., Zhao, Y., Eysel, U. T. & Funke, K. Effect of transcranial magnetic stimulation on single-unit activity in the cat primary visual cortex. *J. Physiol.* 533, 665–679 (2003).
18. MolaZde, V., Giannikopoulos, D., Eysel, U. T. & Funke, K. Paired-pulse transcranial magnetic stimulation protocol applied to visual cortex of anaesthetized cat: Effects on visually evoked single-unit activity. *J. Physiol.* 566, 955–965 (2005).
19. Kammer, T. & Baumann, L. W. Phosphene thresholds evoked with single and double TMS pulses. *Clin. Neurophysiol.* 121(3), 376–379 (2010).
20. Ray, P. G., Meador, K. J., Epstein, C. M., Loring, D. W. & Day, L. J. Magnetic stimulation of visual cortex: Factors influencing the perception of phosphenes. *J Clin Neuropsychol.* 15, 351–357 (1998).
21. Sparring, R. et al. Investigation of the primary visual cortex using short-interval paired-pulse transcranial magnetic stimulation (TMS). *Neurosci. Lett.* 382, 312–316 (2005).
22. Kujirai, T. et al. Corticocortical inhibition in human motor cortex. *J. Physiol.* 71, 501–519 (1993).
23. Fierro, B. et al. 1 Hz rTMS enhances extraverted cortex activity in migraine: Evidence of a reduced inhibition? *Neurology* 61, 1446–1448 (2003).
24. Najib, U., Horvath, J. C., Silvanto, J. & Pascual-Leone, A. State-dependency effects on TMS: A look at motive phosphene behavior. *J. Vis. Exp.* 46, e2273 (2010).
25. Kamitani, Y. & Shimojo, S. Manifestation of scotomas created by transcranial magnetic stimulation of human visual cortex. *Nat. Neurosci.* 2, 767–771 (1999).

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**Author contributions**

H.L., D.W., and S.S. conceptualized the research idea and designed the experiments. H.L., D.W., and N.H. collected the data and conducted the analysis. H.L., D.W. and S.S. wrote the manuscript. S.S. supervised and funded the project.

**Additional information**

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