Early Visual Cortex Stimulation Modifies Well-Consolidated Perceptual Gains

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Abstract
Perception thresholds can improve through repeated practice with visual tasks. Can an already acquired and well-consolidated perceptual skill be noninvasively neuromodulated, unfolding the neural mechanisms involved? Here, leveraging the susceptibility of reactivated memories ranging from synaptic to systems levels across learning and memory domains and animal models, we used noninvasive brain stimulation to neuromodulate well-consolidated reactivated visual perceptual learning and reveal the underlying neural mechanisms. Subjects first encoded and consolidated the visual skill memory by performing daily practice sessions with the task. On a separate day, the consolidated visual memory was briefly reactivated, followed by low-frequency, inhibitory 1 Hz repetitive transcranial magnetic stimulation over early visual cortex, which was individually localized using functional magnetic resonance imaging. Poststimulation perceptual thresholds were measured on the final session. The results show modulation of perceptual thresholds following early visual cortex stimulation, relative to control stimulation. Consistently, resting state functional connectivity between trained and untrained parts of early visual cortex prior to training predicted the magnitude of perceptual threshold modulation. Together, these results indicate that even previously consolidated human perceptual memories are susceptible to neuromodulation, involving early visual cortical processing. Moreover, the opportunity to noninvasively neuromodulate reactivated perceptual learning may have important clinical implications.

Key words: consolidation, perceptual learning, reactivation, reconsolidation, rTMS

Introduction

Improvements in perceptual sensitivity have been observed across species and sensory domains, including the visual system. These processes of visual perceptual learning (VPL) have been documented to occur even well beyond the critical period of development (Karni and Sagi 1991; Censor et al. 2012; Frank et al. 2020). Such changes in perception have been attributed to brain plasticity mechanisms, involving activity changes in early visual areas and their higher level readouts (Karni and Sagi 1991; Poggio et al. 1992; Schoups et al. 1995; Shibata et al. 2014). Moreover, neuroimaging studies in humans revealed increased primary visual cortex (V1) activity as early as 6 h following visual skill acquisition, implicating the involvement of V1 in between-session “offline” learning and consolidation processes (Schwartz et al. 2002; Yotsumoto et al. 2009). Until recently, the dominating perceptual learning dogma dictated that “practice makes perfect” (Karni and Sagi 1991; Censor et al. 2012), meaning, training-induced changes in perception and the neural plasticity mechanisms that are believed to accompany them are the product of prolonged and repeated performance...
or exposure to a certain task or stimulus (Schoups et al. 2001; Schwartz et al. 2002; Chein and Schneider 2005). Recently, it has been shown that brief reactivations of encoded visual memories by a reminder are sufficient to improve perceptual discrimination thresholds, similarly to learning achieved with standard prolonged training (Amar-Halpert et al. 2017). Several mechanisms may account for this reactivation-based learning, one of them being the reactivation–reconsolidation framework predominantly originating from fear conditioning studies at the synaptic level in rodents. According to this concept, even after being consolidated, memories are still dynamic. Meaning, upon retrieval, or reactivation by a reminder, the existing memory becomes susceptible to modulations and can be updated, strengthened, or degraded (Nader et al. 2000; Nader and Hardt 2009; Dudai 2012). Indeed, Bang et al. (2018) have used behavioral interference paradigms to show that reconsolidation operates in perceptual learning. These converging lines of evidence raise the following question: Can an already acquired and well-consolidated reactivated perceptual skill be noninvasively neuromodulated, unfolding the neural mechanisms involved?

Here, leveraging the susceptibility of reactivated memories, we used non-invasive brain stimulation to (1) neuromodulate reactivated VPL and (2) reveal the underlying neural mechanisms. Subjects were trained on a visual texture discrimination task (TDT) (Karni and Sagi 1991) for four daily sessions (Fig. 1). In the fifth session, following a brief reactivation, we used inhibitory repetitive transcranial magnetic stimulation (rTMS) over early visual cortex to test for neuromodulation of the learnt visual skill, resulting in reduced discrimination thresholds. Modulation of perceptual thresholds would indicate that even previously consolidated human perceptual memories are susceptible to neuromodulation. In addition, this would point to early cortical stages of sensory processing being critically involved.

Previous psychophysical results suggest that VPL is specific to the trained retinotopic location, indicating that learning involves neuronal populations in early visual cortex (V1 and V2) representing the trained part of the visual field (Karni and Sagi 1991). This assumption has received additional support by experiments using functional magnetic resonance imaging (fMRI) reporting specific activity changes in the trained retinotopic representations of early visual cortex as a result of VPL (e.g., Schwartz et al. 2002; Furmanski et al. 2004; Yotsumoto et al. 2008, 2009). Based on these results, we hypothesized that VPL in the current task would primarily involve the trained part of early visual cortex. Transcranial magnetic stimulation (TMS) has the spatial precision to inhibit processing in the trained part of early visual cortex while leaving processing in the untrained part of early visual cortex largely intact (e.g., Bang et al. 2019).

There is a high degree of interconnectivity between different retinotopic representations within early visual cortex (Gençet al. 2016), and the strength of this interconnectivity is predictive of subsequent VPL (Baldassarre et al. 2012). Due to the specific nature of the TDT used in the current study, we expected to find highly location-specific VPL. We hypothesized that subjects with a higher degree of location-specific visual processing (as indicated by lower interconnectivity between trained and untrained parts of early visual cortex prior to training) would also show greater interference effects if the trained retinotopic location would be temporarily inhibited by TMS. On the contrary, subjects with higher interconnectivity and greater amounts of shared processing and learning between trained and untrained parts of early visual cortex were expected to better compensate for the effects of inhibitory TMS and thus show less pronounced interference effects. Thus, we predicted a correlation between pretraining interconnectivity between trained and untrained parts of early visual cortex and TMS-induced interference of reactivated VPL across subjects.

Materials and Methods

Subjects

A total of 34 healthy subjects, aged 18–40 years (24 females; mean age 24.3 ± 2.5 years standard deviation (SD), gave their informed written consent to participate in the study, which was approved by the Tel Aviv Sourasky Medical Center and Tel Aviv University’s ethics committees. All procedures were in accordance with approved guidelines of the Helsinki Declaration. Subjects were randomly assigned to the visual and control stimulation groups, which were conducted in a single-blinded fashion. In the visual stimulation group, inhibitory rTMS was applied over early visual cortex. In the control stimulation group, inhibitory rTMS with the same parameters was applied over the vertex. All subjects had normal or corrected-to-normal vision, were not video gamers, did not participate in other visual experiments while participating in the current experiment, and reported at least 6 h of sleep the night before each experimental session (conducted during daytime). Four subjects did not complete the experiment following the initial session, due to repeated fixation and mistyping errors, which prevented reliable measurement of the peripheral discrimination threshold. One subject was excluded due to extreme perceptual threshold changes following vertex rTMS (more than 2 SD away from the group mean). Therefore, there were a total of n = 15 subjects in the visual stimulation group and a total of n = 14 subjects in the control stimulation group.

Experimental Procedure

The study comprised six sessions (Fig. 1a) spaced 1–2 days apart to enable consolidation (Squire 1986). On the first session, subjects first underwent an fMRI resting state scan in which they were instructed to keep their eyes closed and not fall asleep for 8 min, followed by a standard localized scan to determine for each subject the rTMS target site over the left hemisphere of the early visual cortex (Fig. 1c). Note that subjects in the control group with vertex stimulation also completed this localized scan to maintain similar procedures in both subject groups. Following the scans, subjects completed a standard session of the TDT (performed outside the scanner, 252 trials; Karni and Sagi 1991; Fig. 1b) in which the memory was encoded and the discrimination threshold was measured. Subjects then returned for three additional TDT practice sessions, separated 1–2 days apart. The first and the last of these four sessions are referred to as “pre-learning” and “post-learning.” On the fifth session, the encoded memory was briefly reactivated with only five trials (Amar-Halpert et al. 2017). Inhibitory rTMS was administered 5 min following reactivation, over early visual cortex (corresponding to visual areas V1 and V2) or a control vertex location (see below) in different subject groups (Tunovic et al. 2014). Based on previous results (Amar-Halpert et al. 2017; Bang et al. 2018), we predicted that the encoded VPL would be in a plastic state following reactivation. We hypothesized that inhibitory rTMS over early visual cortex should interfere with reactivated VPL and as a result deteriorate subjects’ performance. To test the
interference effect of the stimulation, a final post-rTMS measurement of perceptual thresholds was conducted on the sixth session. This session was identical to the pre-learning and post-learning sessions and is referred to as the "post-rTMS" session. For the control stimulation group no such interference effect was predicted. Rather, subjects in this group were predicted to improve their performance after reactivation (Amar-Halpert et al. 2017).

Visual Task

Subjects performed the standard visual TDT (Karni and Sagi 1991), with a target frame (10 ms) followed by a patterned mask (100 ms). They were asked to discriminate whether a target stimulus (presented at the lower right quadrant of the visual field at 5.71° from screen center) consisting of three diagonal bars, embedded in an array of horizontal bars (19 × 19 elements, 0.58° × 0.04° spaced 0.82° apart, 0.04° jitter), was horizontal or vertical (Fig. 1b). No feedback about response accuracy was provided for this task. Fixation was enforced by a forced-choice letter discrimination task (rotated "L" or "T" at the center of the display) with an auditory feedback (tone for correct discrimination). Display size was 15.6° × 15.1° (viewed from 100 cm, 20° CRT HP p1230 monitor, refresh rate 100 Hz, mean texture luminance 79 cd/m²).

The stimulus was backward masked, and the intervals between the target and the mask stimuli (stimulus onset asynchrony, SOA) were randomly changed between trials. SOAs ranged from 40 to 340 ms (40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 300, and 340 ms). Each session consisted of 18 trials per SOA (total of 252 trials). The reactivation session consisted of five trials at the longest SOA (340 ms). To familiarize the subjects with the task, a pretraining block of 10 trials at 340 ms SOA was conducted before the first session (Censor et al. 2006). This block was repeated until subjects reached 90% correct responses for the peripheral target discrimination (maximum of 10 blocks, after which subjects who did not reach the criterion did not enter the experiment). Pretraining was followed by a short familiarization block of one trial per each SOA (total of 14 trials).

Behavioral Data Analysis

To quantify TDT performance gain, a daily threshold was calculated for each session using the standard Weibull fit for the psychometric curve with slope $\beta$ and finger-error parameter $1-p$ yielding the function:

\[ P(t) = p \left[ 1 - \frac{1}{2} \exp \left[ -\left( \frac{t}{T} \right)^\beta \right] \right] + \frac{1-p}{2} \left[ 1 + p \left[ 1 - \exp \left[ -\left( \frac{t}{T} \right)^\beta \right] \right] \right] \]
where \(t\) is the SOA, \(p\) is the success ratio (in the closed interval \([0,1]\)) of target discrimination for a given SOA, \(T\) is the threshold for each curve, defined as the SOA for which 81.6% of responses were correct when \(p = 1\). To quantify threshold improvements by training, a \(2 \times 2\) mixed analysis of variance (ANOVA) with the within-subject factor of training session (pre-learning vs. post-learning) and the between-subject factor of group (visual stimulation vs. control stimulation) was conducted. Furthermore, we conducted independent sample t-tests between the visual and control stimulation groups on thresholds achieved in the pre-learning and post-learning sessions to test for any differences in thresholds between the two groups before and after VPL. The effects of rTMS on reactivation of VPL were quantified in thresholds between the two groups before and after VPL. The average subject's high-resolution anatomical MRI scan was reconstructed from the FreeSurfer template brain to the reconstructed individual brains. The intersection between brain activity corresponding to the representation of the trained lower right visual quadrant (at a threshold of \(P < 0.001\), false discovery rate corrected) on the inflated left hemisphere and the representation of V1 and V2, as defined by the atlas of Glasser et al. (2016), was used as a region-of-interest (ROI) for rTMS and the functional connectivity analysis (Fig. 3a). The average stimulation location, across subjects, in MNI coordinates was \((x, y, z) = (−13, −94, 15) \pm (1.5, 0.8, 1.9)\) standard error of the mean (SEM). The remaining untrained part of V1 and V2 was used as an additional ROI for the connectivity analysis (Fig. 3a).

The resting state fMRI scans were analyzed by extracting the signal time-courses of the ROIs corresponding to the trained and untrained parts of V1 and V2 in the left hemisphere (averaged across all voxels within the ROIs). Furthermore, the time-courses of the white matter, the ventricles, and the cerebrospinal fluid were extracted as signals-of-no-interest by using a principal component analysis as implemented in the FSFAST processing pipeline for functional connectivity. Next, variance corresponding to the signals-of-no-interest as well as to motion correction parameters and a linear scanner drift were removed from the signal time-courses of the ROIs by multiple regression analysis. After removing this variance, the signal time-courses of the ROIs were correlated for each subject using Pearson’s correlation. The Pearson’s \(r\) correlation scores were transformed into Fisher Z-scores and correlated with the threshold change after rTMS separately for the visual and control stimulation groups, again by using Pearson’s correlation. For a control analysis, the Fisher-transformed functional connectivity scores between trained and untrained parts of early visual cortex were compared between the visual and control stimulation groups by means of independent sample t-test to rule out the possibility that preexisting differences in connectivity strengths between the two subject groups contributed to differential correlation results with the performance changes after rTMS.

### Imaging Data Acquisition

Imaging data were acquired with a 3 T Siemens Magneton Prisma Scanner equipped with a 20-channel receiver head coil at the Alfredo Federico Strauss Center for Computational Neuro-Imaging, Tel Aviv University. High-resolution anatomical images were acquired with an MPRAGE sequence (repetition time/echo time \([\text{TR/TE}] = 1750/2.61\) ms; flip angle \(= 8\); in-plane acquisition matrix \([\text{AM}] = 224 \times 224\); field of view \([\text{FOV}] = 224 \times 224\) mm; slice thickness \(= 1\) mm; 176 axial slices).

Resting state fMRI images and localizer scans to determine the rTMS target site were acquired using the CMRR multiband accelerated echo planar imaging pulse sequence of functional \(T_2^*\)-weighted images \([\text{TR/TE}] = 2000/30\) ms; flip angle \(= 82\); AM \(= 104 \times 104\); FOV \(= 208 \times 208\) mm; slice thickness \(= 2\) mm; 66 interleaved axial slices per volume). The resting state functional scans comprised a total of 240 volumes (acquisition duration \(= 8\) min). The first five volumes were discarded to account for \(T_1\)-equilibrium effects.

The functional localizer scan, which was used to determine each subject’s rTMS administration site, comprised a total of 102 volumes (acquisition duration \(= 3\) min and 24 s) using the same imaging parameters as for the resting state fMRI scan. For this localizer, a checkerboard circle changing color (red–green, green–red, yellow–blue, blue–yellow) every 125 ms was presented to subjects. The circle was presented in the lower right quadrant to match the discrimination task’s target stimulus site. Subjects were instructed to maintain fixation on a cross at the center of the screen during the scan. Blocks with stimulation alternated with blocks of central fixation. Stimulation and fixation blocks were 16 and 15 s long, respectively. The first five volumes were discarded to account for \(T_1\)-equilibrium effects.

### Imaging Data Analysis

The imaging data were analyzed with the FreeSurfer software package including the FSFAST toolbox for functional imaging analysis (Martinos Center for Biomedical Imaging). Each subject’s high-resolution anatomical scan was reconstructed and inflated (Dale et al. 1999; Fischl et al. 1999). Functional imaging data were preprocessed including motion correction, co-registration to the reconstructed high-resolution anatomical scan of the brain, smoothing with a three-dimensional Gaussian kernel (full-width at half-maximum \(= 3\) mm), and intensity normalization.

The preprocessed functional imaging data of the localizer scan were analyzed with a general linear model (GLM) approach. The GLM included two regressors-of-interest for the stimulation and baseline blocks, respectively. Furthermore, motion correction parameters and a linear scanner drift predictor were included as regressors-of-no-interest. The blood-oxygenation-level-dependent response was estimated with the SPM hemodynamical response function. The trained visual quadrant was defined by contrasting activity during stimulation with baseline and was constrained to early visual cortex (V1 and V2). To this aim, V1 and V2 were defined by using the recent atlas of the brain proposed by Glasser et al. (2016). V1 and V2, as defined by this atlas, were remapped from the FreeSurfer template brain to the reconstructed individual brains. The intersection between brain activity corresponding to the representation of the trained lower right visual quadrant (at a threshold of \(P < 0.001\), false discovery rate corrected) on the inflated left hemisphere and the representation of V1 and V2, as defined by the atlas of Glasser et al. (2016), was used as a region-of-interest (ROI) for rTMS and the functional connectivity analysis (Fig. 3a). The average stimulation location, across subjects, in MNI coordinates was \((x, y, z) = (−13, −94, 15) \pm (1.5, 0.8, 1.9)\) standard error of the mean (SEM). The remaining untrained part of V1 and V2 was used as an additional ROI for the connectivity analysis (Fig. 3a).

Non-Invasive Brain Stimulation

One hertz inhibitory rTMS (Dayan et al. 2013) was applied for 15 min, a common protocol that has been shown to affect cortical excitability beyond the duration of the rTMS application itself (Sandrini et al. 2011). Stimulation was applied using a standard figure of eight coil connected to a rapid rate magnetic Magstim stimulator. Stimulation intensity was set to 115% of each subject’s resting motor threshold (RMT) (Chen et al. 1997; Stewart et al. 2001; Deblieck et al. 2008). The RMT was determined by stimulating the left primary motor cortex (identified by using each subject’s high-resolution anatomical MRI scan)
over the area analogous to the movement of the right first dorsal interosseous (FDI) muscle (Censor et al. 2010) with the handle pointing forward at a 45° angle and recording of the FDI muscle electromyogram. RMT was chosen to be the minimum stimulator output required to evoke motor evoked potentials greater than 0.05 mV in at least 5 out of 10 consecutive trials of the same intensity (Rossini et al. 1994). The signal was processed via Signal 6.02 (Cambridge Electronic Design Ltd) and displayed on a monitor in real-time (band-passed from 25 Hz to 1 kHz, sampled at 2 kHz). For the visual stimulation group, inhibitory rTMS was administered over the area within the early visual cortex retinotopically mapped to the lower right quadrant of the visual field as defined by the fMRI analysis (Fig. 1c), with the coil handle pointing left parallel to the horizontal plane. For the control stimulation group, stimulation was applied over the vertex, a reliable control condition, as the evoked auditory and somatosensory activations caused by vertex TMS are similar to those of real TMS (Sandrini et al. 2003, 2011, 2013; Dafotakis et al. 2008; Ko et al. 2008; Nowak et al. 2008; Dayan et al. 2013). The vertex was defined as the point midway between the inion and the nasion and equidistant from the left and right intertragal notches (Pitcher et al. 2007), with the coil handle pointing backward parallel to the mid-sagittal plane. TMS sites were located and kept stable using a frameless stereotactic neuronavigation system (Brainsight2, Rogue Research, www.rogue-research.com). The neuronavigation system was also used to co-register subjects’ heads and their acquired anatomical scans and to mark stimulation targets prior to rTMS. Four landmarks were used for co-registration (nasion, tip of the nose, and left and right crura of helix). Due to minor discomfort, one subject from the control stimulation group did not receive stimulation and one subject from the visual stimulation group received 8 min of stimulation. Both of these subjects completed the experiment and were included in the analysis.

Statistical Analysis

Behavioral and imaging data were analyzed using parametric statistics (mixed design ANOVA, followed by post hoc independent sample t-tests, Pearson correlation). For all statistical tests, the two-tailed α-level was set to 0.05. We report the following measures of effects size for different statistical tests: partial η² for ANOVAs, Cohen’s d for t-tests and r for Pearson correlations.

Results

Initial performance, before VPL, was comparable across the visual and control stimulation groups. An independent sample t-test showed no significant differences in thresholds in the pre-learning session between the groups t(27) = −0.39, P = 0.70, d = −0.15 (Fig. 2a). Furthermore, VPL was comparable across the visual and control stimulation groups. A 2 × 2 mixed ANOVA with the within-subject factor of session (pre-learning vs. post-learning) and the between-subject factor of group (visual stimulation vs. control stimulation) showed no significant main effect of group [F(1,27) = 0.24, P = 0.63, partial η² = 0.01] and no significant interaction between session and group [F(1,27) = 0.02, P = 0.90, partial η² = 0.001], indicating that both groups improved their perceptual thresholds similarly between pre-learning and post-learning (Fig. 2a). There was a significant main effect of session [F(1,27) = 4.54, P < 0.001, partial η² = 0.16], suggesting that perceptual thresholds were significantly lower in the post-learning compared with the pre-learning session in both groups (Fig. 2a). Finally, the achieved thresholds in the post-learning session were not significantly different between both groups (independent sample t-test; t(27) = −0.44, P = 0.67, d = −0.16), indicating that performance after perceptual learning was comparable between groups (Fig. 2a).

Did non-invasive brain stimulation over early visual cortex modify the acquired perceptual skill? A 2 × 2 mixed ANOVA with the within-subject factor of session (post-learning vs. post-rTMS) and the between-subject factor of group (visual stimulation vs. control stimulation) showed a significant interaction between session and group [F(1,27) = 4.96, P = 0.04, partial η² = 0.16], indicating that perceptual thresholds in the visual stimulation group deteriorated after inhibitory rTMS (mean threshold change = −8.27 ± 4.89%, SEM), whereas thresholds in the control stimulation group continued to improve (mean threshold change = +6.47 ± 3.20%, SEM) (Fig. 2b). This was confirmed in a post hoc independent samples t-test, which showed significant differences in percent threshold changes after rTMS between the visual and control stimulation groups t(27) = −2.48, P = 0.02, d = −0.92 (Fig. 2b). There were no significant main effects of session [F(1,27) = 0.04, P = 0.85, partial η² = 0.01] or group [F(1,27) = 0.22, P = 0.64, partial η² = 0.01]. Taken together, these results indicate that inhibitory rTMS over early visual cortex modulated well-consolidated reactivated VPL.

To further examine the involvement of early visual cortex, we tested whether resting state functional connectivity between the trained and untrained parts of early visual cortex prior to training predicted the magnitude of perceptual threshold modulation (Fig. 3). The results of this analysis showed a significant correlation between pre-learning connectivity between trained and untrained parts of early visual cortex and subsequent threshold changes following inhibitory rTMS over early visual cortex in the visual stimulation group (r = 0.54, P = 0.04; Fig. 3b). Specifically, subjects with less pronounced functional connectivity between trained and untrained parts of V1 and V2 showed greater deterioration of their perceptual thresholds after inhibitory rTMS. No such association was observed for the control stimulation group (r = −0.13, P = 0.66; Fig. 3c). These results suggest that subjects with less pronounced functional connectivity between trained and untrained parts of early visual cortex and thus more localized processing exhibited more pronounced interference following inhibitory rTMS over the trained part of early visual cortex. Importantly, the functional connectivity between trained and untrained parts of V1 and V2 per se was not significantly different between the visual and control stimulation groups t(27) = 0.73, P = 0.47, d = 0.27, suggesting that there were no preexisting group-level differences in connectivity strengths between the two subject groups.

Discussion

The results provide novel evidence indicating that an already acquired and well-consolidated reactivated perceptual skill can be non-invasively neuromodulated, unfolding the neural mechanisms involved. Following learning and consolidation of a new visual skill, inhibitory rTMS over early visual cortex deteriorated the visual skill memory, relative to control stimulation. Consistently, resting state functional connectivity between trained and untrained parts of early visual cortex prior to training predicted the magnitude of perceptual threshold modulation. Specifically, subjects with less pronounced functional connectivity and thus more localized early visual processing showed greater deterioration of their perceptual thresholds following rTMS. No such
Figure 2. Behavioral results. (a) Group-level TDT thresholds per session. Red line = visual stimulation group (n = 15) with inhibitory rTMS over early visual cortex after reactivation. Blue line = control stimulation group (n = 14) with vertex stimulation. "Lightening" represents the rTMS stimulation after reactivation in session five. Dashed line represents perceptual threshold changes during initial learning. Solid line represents the rTMS effects. There was a significant interaction between session (four vs. six) and group (visual stimulation vs. control stimulation), such that subjects in the visual stimulation group deteriorated their perceptual thresholds as a result of inhibitory rTMS over early visual cortex, whereas subjects in the control stimulation group improved their thresholds. (b) Group-level TDT performance change before and after rTMS. Left side bars show initial learning. Right side bars show the threshold modulations after reactivation with rTMS in the visual and control stimulation groups. *P < 0.05; error bars are SEM.

Figure 3. Functional connectivity results. (a) Localization of trained and untrained parts of early visual cortex in the left hemisphere of an example subject. The black outline shows early visual cortex (V1 and V2) defined by using an anatomical atlas (see Methods for details). The trained part of early visual cortex corresponds to stronger blood-oxygen-level-dependent (BOLD) activity during visual stimulation in the lower right visual quadrant (color coded in red-yellow) within V1 and V2, whereas the remaining part of V1 and V2 corresponds to untrained early visual cortex. The functional connectivity strength between trained and untrained parts of early visual cortex was measured with resting state functional MRI prior to training in each subject. The strength of this connectivity was used as a predictor for perceptual threshold changes following rTMS. The z-scored time-courses of the BOLD resting state signal in trained and untrained parts of early visual cortex are shown for an example subject. (b) Results for the visual stimulation group (n = 15). Subjects with less pronounced functional connectivity (i.e., lower values on the x-axis) between trained and untrained parts of their early visual cortex showed greater interference effects by inhibitory rTMS after reactivation (i.e., more negative perceptual threshold changes after rTMS, as presented on the y-axis). (c) Results for the control stimulation group (n = 14). No significant association between functional connectivity strength and perceptual threshold change was observed.
association was observed for the control group. These results indicate that even previously consolidated human perceptual memories are susceptible to neuromodulation, involving early visual cortical processing.

Previous research has pointed to the involvement of V1, where neurons show strong orientation tuning and retinotopic specificity, in consolidation of VPL (Karni and Bertini 1997; Schoups et al. 2001; Schwartz et al. 2002; Furmanski et al. 2004; Yotsumoto et al. 2008, 2009). Evidence has been provided supporting long-lasting plasticity in V1 through consolidation processes of newly gained orientation discrimination skills (Fourtois et al. 2008) and reconsolidation of visual perceptual memories (Bang et al. 2018). Of note, post-training synaptic changes in the visual network are not likely to be limited to lower level areas as those are coupled with higher level regions during consolidation in both early and advanced stages of VPL (De Weerd et al. 2012). Our finding that rTMS interference with early visual processing following memory reactivation did not fully degrade the initial memory but rather resulted in a moderate modulation of VPL is consistent with the above theories. Therefore, our results point to the importance of early visual cortex in modulations of previously learned visual skills, but may also suggest further involvement of higher level regions in this process (De Weerd et al. 2012).

Our results also show that the connectivity strength between trained and untrained parts of early visual cortex, as measured by resting state fMRI prior to training, is predictive of the magnitude of subsequent rTMS interference following reactivation. Specifically, subjects with less pronounced connectivity and thus more localized processing exhibited greater interference effects. This result supports the hypothesis that the reactivation mechanism involves early stages of visual cortical processing (Karni and Sagi 1991; Schoups et al. 2001; Schwartz et al. 2002; Furmanski et al. 2004; Yotsumoto et al. 2008, 2009; Bang et al. 2018, 2019), where neurons exhibit preferences for retinotopic locations and where processing is more compartmentalized. The association of reactivation in VPL with early cortical stages of sensory processing also resembles results obtained in other procedural learning systems. For example, it has been shown that inhibitory rTMS over the primary motor cortex inhibits cortical excitability and can interfere with reactivated motor memories (Censor et al. 2010). Of note, our vertex control condition was even stricter than a reactivation alone condition, which is often utilized, further controlling for the topographic specificity of the rTMS effects, as commonly performed in previous studies (Ruff et al. 2006). Similarly, independent stimulation, which is unpaired with behavior, does not result in long-term effects (Pascual-Leone et al. 1998; Lambon Ralph et al. 2008). Nevertheless, further studies are required to understand potential relations to mechanisms such as reactivation–reconsolidation processes (Elsey et al. 2018) and to determine whether facilitatory rTMS protocols could enhance reactivated perceptual memories, gaining better understanding of the mechanisms underlying VPL.

Although not directly tested in the current study, it would be interesting to investigate whether rTMS also affected tasks other than the TDT, involving the same cortical region. We hypothesize that this would not be the case. First, previous studies have shown that interventions paired with reactivation specifically affected the reactivated skill or memory. This is consistent with the memory reconsolidation account, according to which upon reactivation the existing memory (rather than nonreactivated memories) becomes susceptible to modulations (Nader and Hardt 2009; Dudai 2012; Lee et al. 2017). Second, as mentioned above, independent stimulation, which is unpaired with behavior (in our case tasks other than the reactivated TDT), does not result in long-term effects (Pascual-Leone et al. 1998; Lambon Ralph et al. 2008).

Overall, this study provides evidence for the role of early visual cortex in neuromodulation of well-consolidated reactivated human VPL, combining behavioral, brain stimulation, and neuroimaging paradigms. The finding that even previously consolidated human perceptual memories are susceptible to neuromodulation may facilitate opportunities to non-invasively neuromodulate human learning utilizing the memory reactivation framework. Consequently, identifying the underlying mechanisms of human memory modification may pave the way for new methods of clinical treatment of conditions involving memory deficits. Over time, better understanding of skill memory dynamics may yield the potential to improve memory gains and prevent forgetting.

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Notes

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