

Causal role of the prefrontal cortex in top-down modulation of visual processing and working memory

Theodore P Zanto, Michael T Rubens, Arul Thangavel & Adam Gazzaley

Selective attention filters information to limit what is encoded and maintained in working memory. Although the prefrontal cortex (PFC) is central to both selective attention and working memory, the underlying neural processes that link these cognitive abilities remain elusive. Using functional magnetic resonance imaging to guide repetitive transcranial magnetic stimulation with electroencephalographic recordings in humans, we perturbed PFC function at the inferior frontal junction in participants before they performed a selective-attention, delayed-recognition task. This resulted in diminished top-down modulation of activity in posterior cortex during early encoding stages, which predicted a subsequent decrement in working memory accuracy. Participants with stronger fronto-posterior functional connectivity displayed greater disruptive effects. Our data further suggests that broad alpha-band (7–14 Hz) phase coherence subserved this long-distance top-down modulation. These results suggest that top-down modulation mediated by the prefrontal cortex is a causal link between early attentional processes and subsequent memory performance.

Selective attention describes goal-directed behavior achieved by orienting the focus of conscious awareness toward relevant stimuli and away from irrelevant or competing stimuli¹. Working memory is the cognitive operation that underlies our ability to temporarily maintain and manipulate attended information in mind when it is no longer accessible in the environment to guide behavior². The functional overlap between selective attention and working memory is intuitive, such that attended items are more likely to be remembered than ignored items. The reverse dependency has also been documented, as selective attention seems to utilize an internal template³ or attentional trace⁴ that is based on working memory to resolve competition among multiple elements in the environment. However, only recently has empirical research begun to identify overlapping neural mechanisms engaged during both selective attention and working memory tasks^{5,6}. Despite evidence suggesting a shared neural substrate between these two cognitive operations, a direct causal link via a common control region and underlying neural process has yet to be established.

The neural process of top-down modulation serves as a fundamental physiological mechanism for selective attention. It underlies our critical ability to selectively focus our attentional resources on relevant stimuli and ignore distractions. It is a bi-directional process, accomplished by differentially enhancing and suppressing neural activity in sensory cortical regions on the basis of the relevance of information to our goals^{7,8}. By generating neural contrast via the enhancement and suppression of activity, top-down signals are also thought to bias the likelihood of successfully representing and maintaining relevant information in a competitive system^{9–11}. Recent neuroimaging studies have provided indirect evidence that successful visual working memory performance in the setting of distracting information (that is, requiring selective attention) is associated with top-down

modulation of activity in visual cortices during early visual processing stages^{12,13}. However, direct evidence that selective attention and working memory are bound by this common neural mechanism has not yet been documented.

Neural network communication has been proposed to be the basis by which top-down modulation is achieved. Evidence in support of this comes from tract-tracing studies in monkeys that identified an anatomical framework of reciprocal cortico-cortical projections between areas in the parietal, frontal and visual association cortices^{14,15}. In addition, functional neuroimaging studies in humans have revealed that top-down modulation during visual processing engages this frontal-parietal-visual network^{7,16,17}. Functional connectivity analysis offers further evidence that the prefrontal cortex (PFC) is a source of this activity modulation¹⁸. Lesion studies in humans¹⁹ and, more recently, transcranial magnetic stimulation (TMS) used to perturb function in frontal and parietal regions provide causal evidence that these areas are a source of top-down activity modulation in visual cortex^{20–23}. However, the mechanism underlying this long-distance communication and its influence on working memory performance has not been evaluated from a causal perspective.

Recent research has identified a region in the PFC, the inferior frontal junction (IFJ), which, on the basis of functional magnetic resonance imaging (fMRI) functional connectivity analysis, may serve as a source of top-down modulation underlying attention to visual features (that is, color and motion)²⁴. Furthermore, electroencephalographic (EEG) data suggest that the IFJ may exert an influence on visual processing as early as 100 ms post-stimulus onset (during the P1 component of the event-related potential, ERP). This time point has previously been shown to be modulated by attention to color and motion stimuli^{25,26}, as well as being related to subsequent

Departments of Neurology, Physiology and Psychiatry, University of California, San Francisco, San Francisco, California, USA. Correspondence should be addressed to T.P.Z. (theodore.zanto@ucsf.edu) or A.G. (adam.gazzaley@ucsf.edu).

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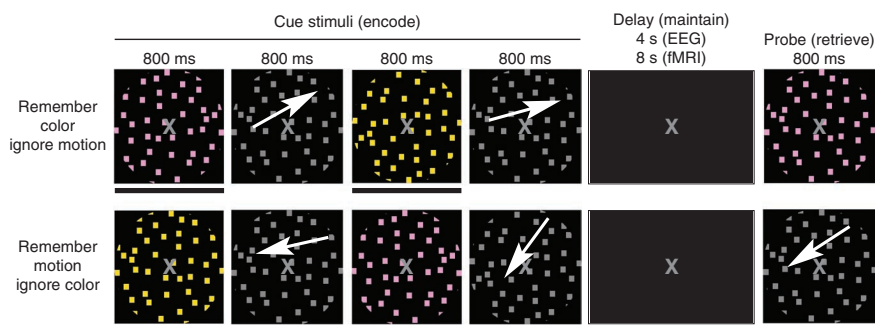


Figure 1 Experimental procedure. White arrows indicate motion and were not present during the experiment. A black bar below a cue stimulus (also not present during the experiment) indicates that it is an item to be remembered. The pictures approximate stimuli appearance (see Online Methods).

working memory performance¹². Moreover, we recently found that top-down modulation may be subserved by long-distance alpha-band phase coherence²⁴, consistent with the suggestion that alpha phase synchronization coordinates top-down control and access to memory traces²⁷. However, this hypothesis has not been directly addressed with a methodology that permits causal inferences.

We directly addressed both the causal role and mechanisms of PFC-mediated top-down modulation, as driven by selective attention, on subsequent working memory performance. This was accomplished by perturbing function in each participant's IFJ via repetitive TMS (rTMS) before they performed a selective-attention, delayed-recognition task and recording the consequences with electroencephalography. The procedure used in this two-session experiment required participants to selectively attend to relevant visual features of sequentially presented stimuli (motion or color), ignore the irrelevant stimuli and maintain the attended features until the information was probed (Fig. 1). The first session used fMRI to identify neural networks associated with top-down modulation. On the basis of each participant's functional connectivity data, the right IFJ was targeted for 1-Hz rTMS during the second session to disrupt the network subserving top-down modulation. Immediately following rTMS, participants performed the same experimental task as in the first session while EEG and working memory performance data were acquired. We assessed the rTMS influence on posterior P1 amplitude modulation and fronto-posterior alpha phase-coherence modulation during stimulus presentation, as well as the effect on working memory accuracy. Our goals were to determine the extent to which early top-down activity modulation in visual cortices was driven by the IFJ and how this affected working memory performance, as well as identify neural mechanisms underlying the long-distance modulatory influences.

RESULTS

Neural networks subserving top-down modulation

Areas V4 and V5/hMT+ are selectively responsive to color and motion features, respectively²⁸, and activity in each region is modulated by top-down attentional processes²⁹. Thus, color and motion features were used as the stimuli during a selective-attention, delayed-recognition task^{12,24} to induce top-down modulation in the visual association cortex (Fig. 1). During the first session, areas V4 and V5 were identified as regions of interest (ROIs) for each participant and we found that they were modulated by attention during the task (Supplementary Fig. 1 and Supplementary Results).

To identify neural networks involved in top-down modulation, we conducted functional connectivity analysis using the beta series correlation approach^{30,31} on the fMRI data using V4 and V5 ROIs

as seed regions. Four group-level network connectivity maps were generated for the encoding period regressor by correlating each seed region with voxels from the rest of the brain during either the congruent or incongruent condition: V4 attend network (V4 seed and remember color condition), V4 ignore network (V4 seed and remember motion condition), V5 attend network (V5 seed and remember motion condition) and V5 ignore network (V5 seed and remember color condition). Connectivity maps were submitted to a repeated measures analysis of variance (ANOVA) with ROI (V4 / V5) and relevance (attend or ignore feature) as factors and corrected for multiple comparisons by

thresholding *P* values with a cluster extent determined by a Monte-Carlo simulation, resulting in a corrected *P* value of 0.01.

We observed main effects for ROI and relevance, as well as an ROI × relevance interaction, in widespread fronto-parietal regions. To further explore the neural networks underlying top-down modulation, we used paired *t* tests to contrast attend and ignore network maps for each seed region. This functional connectivity analysis revealed that fronto-parietal neural networks were engaged during top-down modulation for both color (Supplementary Table 1) and motion features (Supplementary Table 2). Although the color and motion top-down network maps were largely distinct from each other, the right inferior frontal junction (IFJ) in the PFC was a node in both color and motion neural networks (Fig. 2). This finding replicates recent results from a separate cohort of participants engaged in the same task, which suggested that the right IFJ acts as a putative control region for long-distance top-down modulation of both color and motion feature processing²⁴. Thus, the right IFJ region served as the target site for rTMS in the second experimental session. Of note, our data and data from a previous study²⁴ both indicate that bilateral IFJ engagement occurs only in the motion network. We hypothesized that rTMS to the right IFJ would affect neural measures and working memory performance more for the remember color condition than for the remember motion condition, as color processing used only the right IFJ and motion processing recruited bilateral IFJ. This prediction served as an internal control of the specificity of rTMS effects.

Effects of rTMS on top-down modulation and working memory

During the second session, we applied 1-Hz rTMS for 10 min to the right IFJ to perturb function in this region³², potentially affecting the neural

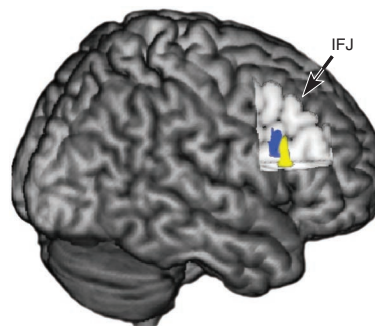


Figure 2 Functional connectivity analysis revealed a fronto-parietal region associated with both motion and color top-down modulation, the right IFJ. However, distinct subregions in the IFJ distinguish motion (blue area) from color (yellow area) networks.

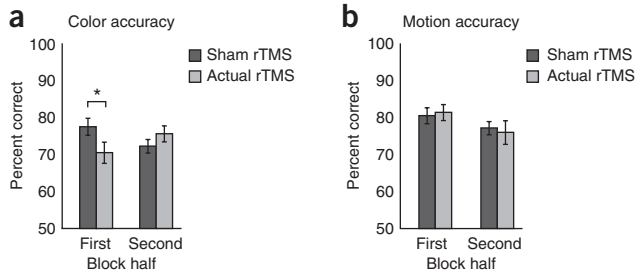


Figure 3 Behavioral results. (a,b) Working memory accuracy for the remember color (a) and remember motion (b) conditions. Accuracy declined as a result of rTMS only for color working memory during the first half of the experimental block. Error bars represent s.e.m. * $P < 0.05$.

network underlying top-down modulation of activity in visual regions and subsequent working memory performance. Each participant received two blocks of rTMS (one preceding each condition), as well as two blocks of sham rTMS (coil angled 90 degrees away from head as a control) followed by 64-channel EEG recordings while they were engaged in the experimental procedure. rTMS effects decrease over time³³; thus, data from each experimental block was divided in half for analysis. We hypothesized that rTMS-related changes in neural activity and working memory performance would be most prominent during the first half of a block. This prediction served as another important internal control.

Measures of working memory accuracy were submitted to a repeated-measures ANOVA with condition (remember color/motion), rTMS (sham/actual) and block half (first/second) as factors. We found a main effect for condition ($F_{1,18} = 7.21, P < 0.05$), a two-way interaction between rTMS and block half ($F_{1,18} = 6.31, P < 0.05$), and a three-way interaction ($F_{1,18} = 5.62, P < 0.05$). *Post hoc* paired *t* tests evaluating the three-way interaction indicated that working memory accuracy for the remember color condition decreased following actual rTMS relative to sham rTMS levels during the first half of the experimental block ($t_{18} = 2.29, P < 0.05$; **Fig. 3a**). However, this effect was not observed during the second half of the remember color condition ($P > 0.1$). Moreover, rTMS did not affect working memory performance during either half of the remember motion condition ($P > 0.5$, each comparison; **Fig. 3b**), indicating that the right IFJ has varying importance based on stimulus feature type. The findings confirmed our predictions that rTMS (and not sham rTMS) would affect working memory performance more during the remember color than the remember motion condition with a greater influence during the first half of the block.

We previously identified the P1 amplitude of the ERP (at posterior electrodes) in response to stimulus presentation during the encoding period as a neural marker of top-down modulation for visual features^{12,25}. To confirm the utility of the P1 amplitude as a marker of color and motion feature processing, we conducted source localization

on the P1 from each attended feature following sham rTMS. We found that the striate and extrastriate visual cortex and portions of the inferior temporal cortex were the source of the P1 component. Notably, these sources included V4 when attending to color and V5 when attending to motion (**Supplementary Fig. 2**). Thus, our initial analysis of the EEG data focused on P1 amplitude modulation and assessed whether it was altered by rTMS.

Measures of top-down modulation using P1 amplitude differences (attend – ignore) were submitted to a repeated-measures ANOVA with stimulus (color/motion), rTMS (sham/actual) and block half (first/second) as factors. We found a main effect for rTMS ($F_{1,18} = 4.58, P < 0.05$) and a three-way interaction ($F_{1,18} = 4.51, P < 0.05$). *Post hoc* paired *t* tests evaluating the three-way interaction revealed that, during the first half of the remember color condition, the neural signature of top-down modulation at the P1 decreased following actual rTMS relative to sham stimulation ($t_{18} = 3.00, P < 0.01$; **Fig. 4a**). The magnitude of P1 modulation during the second half of the remember color condition was not different between actual and sham rTMS ($t_{18} = 0.86, P > 0.4$; **Fig. 4b**). These findings indicate that the magnitude of top-down modulation for color processing was decreased as a result of rTMS only during the first half of the experimental block, comparable to the working memory accuracy findings. *Post hoc* paired *t* tests of P1 modulation during the remember motion condition showed no differences between sham and actual rTMS during either the first half ($t_{18} = 0.43, P > 0.6$; **Fig. 4c**) or second half ($t_{18} = 1.93, P > 0.05$; **Fig. 4d**) of the experimental block. These results were comparable to the lack of rTMS effects on working memory accuracy for motion and further confirm our prediction that neural measures of color processing are more affected by rTMS than motion processing with a greater influence during the first half of the block.

To explore whether the observed changes in P1 modulation during the first half were the results of changes in enhancement of relevant stimuli or suppression of irrelevant stimuli, we directly compared data from sham and actual rTMS. We found that rTMS reduced the degree to which ignored color stimuli were suppressed ($t_{18} = 2.43, P < 0.05$).

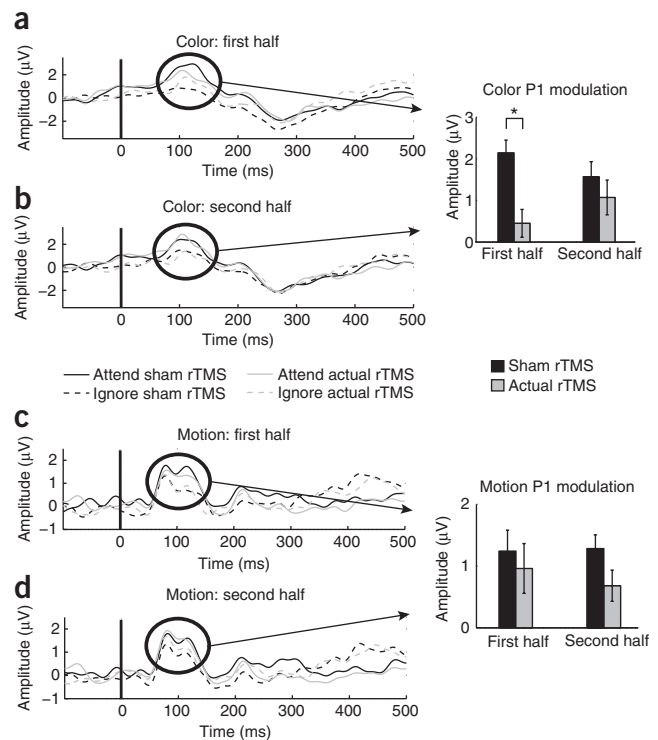


Figure 4 Attentional modulation of the P1 during color processing and motion processing. (a) P1 modulation substantially declined during the first half of the experimental block following actual rTMS compared with sham. (b) Attentional modulation was observed during the second half of each block following both sham and actual rTMS. Bar graphs indicate the magnitude of attentional modulation (attend – ignore). rTMS altered the magnitude of P1 attentional modulation only during the first half of the actual rTMS block. * $P < 0.05$. (c,d) Attentional modulation of the P1 during motion processing was observed during both the first and the second half of each experimental block. rTMS did not change the magnitude of attentional modulation to motion stimuli. Error bars represent s.e.m.

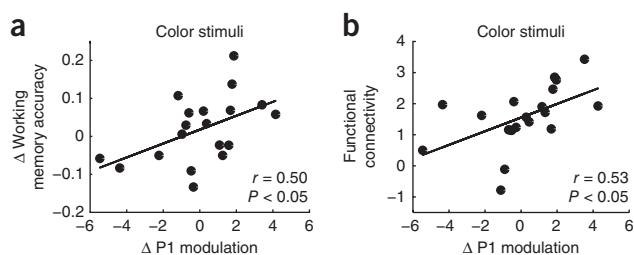


Figure 5 Neuro-behavioral correlations. (a,b) Regression between the rTMS-related change in P1 modulation and either working memory accuracy (a) or V4/IFJ functional connectivity (b) when attending to color. Δ = sham – actual rTMS.

In addition, an rTMS-related decrease in the amount relevant color stimuli was enhanced strongly trended toward significance ($t_{18} = 1.95$, $P = 0.067$). Thus, the rTMS-induced decrease in top-down modulation was a result of combined effects of a decline in the participant's ability to enhance P1 activity to relevant color stimuli and suppress P1 activity to irrelevant color stimuli. This implies that a single region in the PFC drives increases and decreases in cortical activity of a distant sensory region solely on the basis of task goals.

To assess whether the absence of a right IFJ rTMS effect for the remember motion condition was a result of bilateral IFJ control (as suggested by the fMRI connectivity data), we split participants into two groups based on the magnitude of left IFJ-V5 functional connectivity in the attend network. Measures of P1 modulation from the posterior left hemisphere during the first half of the remember motion condition were submitted to an ANOVA with subgroup (higher left IFJ-V5 connectivity, lower left IFJ-V5 connectivity) and rTMS (sham rTMS, actual rTMS) as factors. We found a significant subgroup \times rTMS interaction ($F_{1,16} = 4.21$, $P = 0.05$). *Post hoc t*-tests revealed no significant P1 modulation difference between sham and actual rTMS for the higher left IFJ-V5 connectivity group ($t_8 = 0.61$, $P > 0.5$), whereas the lower left IFJ-V5 connectivity group displayed reduced P1 modulation following actual rTMS ($t_8 = 3.89$, $P < 0.005$). Direct comparisons between the higher and lower left IFJ-V5 connectivity groups revealed no difference following sham rTMS ($t_{16} = 1.32$, $P > 0.2$), but for actual rTMS, lower left IFJ-V5 functional connectivity was associated with diminished P1 modulation compared with higher left IFJ-V5 connectivity ($t_{16} = 2.14$, $P < 0.05$). This supports our hypothesis that bilateral IFJ control compensates for the disruptive effects of rTMS to the right IFJ for motion processing.

Previous findings have also identified attentional modulation in posterior electrodes to stimulus features at later processing stages, reflected as the selection negativity (200–400 ms post-stimulus onset)^{25,34}. Mean amplitudes of the selection negativity were submitted to a repeated-measures ANOVA with stimulus (color/motion), rTMS (sham/actual) and block half (first/second) as factors. We found no main effects or interactions. Thus, the right IFJ rTMS effects on activity modulation appear to be limited to early visual processing, highlighting how rapidly frontally dependent biasing of posterior areas may occur¹⁹.

Correlations between EEG, fMRI and behavioral data

To further elucidate the relationship between IFJ control of top-down modulation and working memory performance, we conducted an across-participant regression analysis on indices of rTMS effects on P1 modulation and working memory accuracy for the remember color condition. P1 modulation was measured as the difference between attended and ignored stimuli. Indices of the rTMS-induced change in P1 modulation and working memory accuracy were calculated as the

difference between sham and actual rTMS (sham – actual rTMS). The analysis for color stimuli revealed that participants who experienced greater P1 modulation decreases following right IFJ rTMS (larger index values) exhibited greater working memory accuracy decline (larger index values) ($r = 0.50$, $P < 0.05$; Fig. 5a). The same analysis for motion stimuli displayed no such relationship ($r = 0.12$, $P > 0.6$). These data strongly suggest that disrupting neural networks subserving top-down modulation results in decreased working memory performance.

We targeted the right IFJ for rTMS because the fMRI functional connectivity analysis identified it as being involved in top-down modulation during both color and motion processing. rTMS to the right IFJ induced a decline in P1 modulation in posterior brain regions for color stimuli during the first half of the experimental block, which was in turn directly related to decrements in working memory performance. We therefore explored the relationship between rTMS-induced changes in P1 modulation for color stimuli and the magnitude of IFJ-V4 fMRI functional connectivity when attending to color stimuli. We found that participants who displayed stronger measures of functional connectivity exhibited greater rTMS-induced declines in P1 modulation ($r = 0.53$, $P < 0.05$; Fig. 5b), suggesting that those who engaged the right IFJ more for top-down modulatory control of color processing were more affected by rTMS to that region.

Phase coherence subserving top-down modulation

Thus far, our data suggest that the right IFJ is functionally connected to visual cortical regions during a selective working memory task and that perturbing right IFJ function with rTMS disrupts activity modulation in posterior cortical areas, as well as subsequent working memory performance. Although these data suggest that rTMS disrupts the neural network subserving top-down modulation, they do not provide a direct measure of the disruption of functional connectivity between frontal and posterior cortices. We recently identified anticipatory signatures of neural modulation for color stimuli as alpha-band phase coherence between electrodes located around the right IFJ and posterior regions²⁴. We therefore explored anticipatory phase coherence (–200 to 0 ms post stimulus onset) in the alpha (7–14 Hz), beta (14–30 Hz) and gamma (30–50 Hz) bands as markers of functional connectivity underlying top-down modulation.

Alpha-band phase coherence was assessed between right-frontal and central-posterior regions, whereas beta and gamma bands were assessed between right-frontal and right-posterior regions, as these

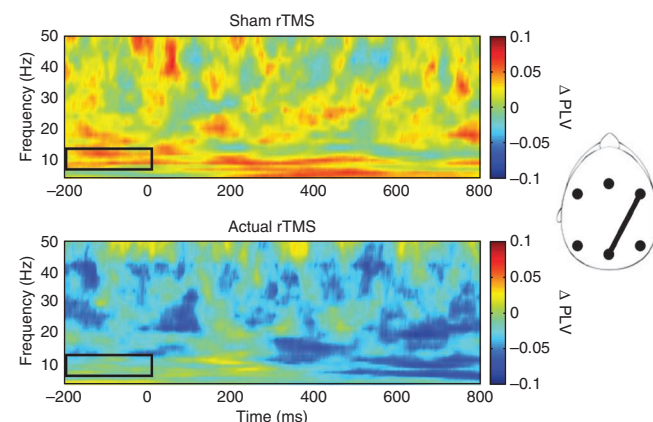


Figure 6 Alpha-band phase coherence between right-frontal and central-posterior regions immediately preceding the onset of color stimuli. Time-frequency maps depict the modulation (attend – ignore) of the phase locking values (PLVs) following sham (top) and actual rTMS (bottom). Black boxes highlight the time-frequency ROI.

regions exhibited the greatest attentional modulation when collapsed across conditions and rTMS treatment. The data were split in half by experimental block to evaluate the decay of rTMS effects and the analysis focused on the color condition. The magnitudes of the phase locking value (PLV) modulation (attend – ignore) were submitted to repeated measures ANOVA with rTMS (sham/actual) and block half (first/second) as factors. In the alpha band, a main effect of rTMS ($F_{1,18} = 4.57, P < 0.05$) indicated that the magnitude of PLV modulation decreased following actual rTMS (Fig. 6 and Supplementary Fig. 3). No other main effect or interaction was observed in the alpha band. In addition, no main effects or interactions were observed in the beta and gamma bands. Although an interaction between rTMS and block half was not observed, the alpha band results are similar to the P1 modulation findings and indicate that anticipatory alpha-band phase coherence may serve as a mechanism for top-down modulation to operate over long cortical distances.

DISCUSSION

We disrupted function in a PFC control region, the right IFJ, with rTMS and assessed the consequences on top-down modulation in posterior cortical regions and working memory performance. We found that top-down modulation during very early visual processing stages of the memoranda and subsequent working memory performance were causally related. There are four results supporting this conclusion. First, color processing showed an rTMS-related decline in both P1 modulation and working memory accuracy. Second, as the P1 modulation recovered with time (that is, in the second half of the block), so did working memory performance. Third, on an individual participant basis, the rTMS-induced effect on P1 modulation during color processing predicted changes in working memory accuracy. Finally, motion processing, which exhibited bilateral connectivity, did not have rTMS-related effects on P1 modulation or working memory accuracy. Thus, we conclude that early top-down activity modulation during stimulus processing driven by attentional demands is causally related to subsequent working memory performance (see Supplementary Discussion). Notably, our data revealed that the IFJ acts as a PFC control region that mediates this causal connection between top-down modulation in the service of attentional goals and subsequent working memory performance. Moreover, top-down modulation may be subserved by alpha phase coherence for long-distance communication.

Although functional connectivity analysis is informative in characterizing neural networks that may be involved in top-down modulation, it is a correlational measure and cannot be used to make strong statements of causality. In recent years, studies coupling rTMS and physiological recordings have provided causal evidence in humans that fronto-parietal regions are a true source of top-down modulation of activity in sensory cortical areas. However, only a few of the fronto-parietal regions proposed to be cognitive control regions underlying top-down modulation have been evaluated with this approach. Studies coupling TMS and neuroimaging has shown that visual ERPs are under top-down influences from frontal eye fields^{20,35} and posterior parietal cortex (PPC)³⁶. Top-down influences operate on areas V1 through V5 and have differential roles based on whether frontal (frontal eye field) or parietal (interparietal sulcus) cortex is the source of modulation²². Research in the last 2 years has indicated that neural modulation occurs early during visual attention to features (~100 ms after stimulus onset)^{12,26}. We found that the right IFJ modulates early visual cortical processing. Specifically, both enhancement and suppression of the P1 amplitude for relevant and irrelevant color stimuli, respectively, were diminished post-rTMS. This suggests that suppression is not simply

the lack of enhancement. Indeed, recent research has shown a selective decline in older adults' ability to suppress irrelevant information, suggesting that enhancement and suppression rely on distinct mechanisms³⁷. An interesting question remains as to how one frontal region may be involved in both enhancing and suppressing posterior neural activity solely on the basis of the goals of the task. One possibility is that functional segregation of the IFJ may exist at a scale smaller than the area affected by rTMS, consistent with the notion of a topographical organization and functional specialization of this region³⁸.

Despite the largely distinct fronto-parietal networks associated with attention to color and motion features, the right IFJ was common to both networks (albeit neighboring subregions in the IFJ). Recent studies have shown that the IFJ is involved in many different tasks, including task switching, interference control and working memory³⁹. As such, it has been hypothesized that the IFJ is involved in updating relevant task representations⁴⁰. Our data support this hypothesis and extend it by suggesting that updating relevant task representations may occur via goal-directed biasing of neural activity in distal cortical regions. Furthermore, we found that this neural biasing optimizes working memory performance. Notably, the IFJ and other frontal cortical regions display topographic organization and functional specialization across multiple spatial tasks, including memory-guided saccades, spatial working memory and finger pointing³⁸. Thus, spatial encoding cannot be discounted as an alternative hypothesis for the proposed role of the IFJ. However, given the many different tasks that have now been shown to utilize the IFJ that do not rely on spatial information^{39,40}, it seems more plausible that the IFJ has a generalized role in updating task representations. This is not to imply a lack of topographical organization in the IFJ. Color and motion processing engage ventral and dorsal visual processing streams, respectively, and this domain specificity is thought to be maintained in frontal regions⁴¹. Our data replicate our previous report that IFJ activity during color processing is localized ventrally in the IFJ relative to regions engaged for motion processing²⁴, thereby supporting selective topographical organization and functional specialization of this region.

Although long-distance cortical communication seems likely to occur via phase information⁴², evidence that top-down modulation utilizes such a mechanism has only recently emerged. Alpha band activity appears to be a prime candidate for such processes, as it is modulated by attention⁴³ and linked to fronto-parietal networks subserving attention²¹ and working memory⁴⁴. We found that pre-stimulus alpha-band phase coherence between frontal and posterior regions is modulated by attention and that perturbing function in the IFJ disrupts this modulation. These results support recent findings that top-down modulation mediated by PFC networks bias activity in sensory cortical regions before stimulus onset^{24,45} and suggest that phase coherence is an operational mechanism. Given that all stimuli in each trial were presented randomly, anticipatory top-down modulation must have occurred as a cognitive set, as opposed to switching between enhancement and suppressive modes. A recent review has suggested that alpha phase synchronization may be interpreted in terms of processes that coordinate top-down control and access to memory traces²⁷. Here, we provide direct evidence to support this hypothesis by showing that alpha phase coherence is modulated by attention and that IFJ rTMS disrupts alpha phase-coherence modulation.

On the basis of these results and previous research, we hypothesize that, when participants are instructed to remember color, the right IFJ biases visual cortical regions via alpha phase coherence before stimulus onset. Anticipatory biasing allows visual information (that is, color features) to be enhanced or suppressed early in the visual processing stream, depending on their relationship to task goals. This contrast between enhanced and suppressed activity yields increased fidelity of

the representation of the memoranda, which leads to improved working memory performance. We identified P1 modulation as a bridge between selective attention and working memory performance and our results support the supposition that selective attention and working memory encoding may not be dissociable at the neural level⁴⁶. However, this may not be true in all cases. Attention operates during both perceptual and post-perceptual stages of stimulus processing, and its influence on working memory may depend on the timing of activity modulation, as well as the type of attention and working memory task⁴⁷. Although our observations were made during the encoding period, this does not preclude the idea that top-down modulation during other stages of the task (for example, the delay period) influences working memory performance. Furthermore, it should be noted that the IFJ is only one region identified in widespread fronto-parietal neural networks used by selective color and motion processing. Future research will assess the necessity of other putative control regions in the frontal and parietal cortex.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/natureneuroscience/>.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

T.P.Z., A.T. and A.G. conceptualized and designed the task. T.P.Z. and M.T.R. performed the experiment. T.P.Z. analyzed the data. T.P.Z. and A.G. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Participants. 20 healthy individuals (mean age = 24.25 years, range = 18–31 years, 7 males) participated in the experiment. All participants gave informed consent to engage in the study according to procedures approved by the Committee for Human Research at the University of California. Each participant had normal or corrected to normal vision. One participant was excluded from all data analysis as a result of excessive artifacts.

Stimuli. The stimuli consisted of a circular aperture of 290 dots ($0.08^\circ \times 0.08^\circ$ each) that subtended 8° of visual angle centered at the fovea. Two types of dots were used during the experiment: gray and moving coherently at 10° per s or stationary and colored along the tritan axis. Stimuli were presented with a gray fixation cross in the center of the circular aperture on a black background. All colored and gray dots were equated for brightness by minimizing heterochromatic flicker in tests carried out before the experiment for each subject. After all stimuli were equated for brightness, participants engaged in two thresholding procedures (one for motion, one for color) to minimize perceptual discriminability differences between participants^{13,25}. A staircase procedure required participants to determine whether two stimuli (directions of motion or colors) were different from each other. The two stimuli were presented for 800 ms each and separated by 2,000 ms. The procedure continued until a 'just 100%' level of performance was reached, meaning that if the stimuli were any more similar, performance would drop below 100%. Thresholding determined the 12 possible directions of motion (three per quadrant, cardinal axes excluded) and the six possible colors each participant received during the experiment.

Several steps were taken to minimize the role of verbalization during feature encoding. Colors were selected from the tritan axis that were not easily nameable and the directions of motion never fell along a cardinal axis to prevent them being verbalized as left, right, up or down. Moreover, all stimuli were selected using a thresholding procedure based on the participant's ability to discriminate color and motion differences. Thus, participants who may have tried using verbalization as a strategy would find it difficult to attribute specific labels to subtle differences between the stimuli. In addition, a post-experimental questionnaire specifically asked what type of strategy the participant employed during each task. Only a few participants reported attempting a verbal strategy, whereas the majority reported using a mental imagery technique. Lastly, analysis focused on early measures of perceptual processing (for example, P1), which precedes typical neural signatures of semantic processing (for example, N400).

Experimental procedure. The experimental procedure is depicted in **Figure 1**, as previously reported¹². Participants were required to either remember the two directions of motion (ignore the two colors) or remember the two colors (ignore motion). Each condition was presented in two blocks and the four blocks were randomized across the experiment and participants. Prior to beginning each block, participants were given task instructions. In addition, a brief (1 s) task reminder was provided at the start of each trial during the EEG session. Both conditions required viewing four sequentially presented images: two differently colored stimuli and two different directions of motion. Every image was presented for 800 ms with a 1,200-ms interstimulus interval (ISI, an 800-ms ISI was used in the EEG experiment). After the four images were presented, there was an 8-s delay (4 s in electroencephalography) followed by a probe stimulus (800-ms duration). Participants responded with a button press whether the probe matched any of the items held in memory. Participants responded by pressing one of two buttons. One half of the probe stimuli matched a previously attended object. Participants were instructed to respond as quickly as possible and to retain accuracy during all conditions. Prior to beginning the experiment, participants were given 12 practice trials for each of the two conditions, split into two blocks (six trials each). During the experiment, participants received 30 trials per condition (60 trials per EEG condition). The stimuli for each trial were randomly selected from pre-determined sets of stimuli, constraining the directions of motion to one quadrant.

fMRI. All fMRI data were collected on a Siemens 3T MAGNETOM Trio with stimuli presented on an LCD monitor positioned behind the head of the participants and viewed using a mirror that was rigidly attached to a 12-channel head-coil. Echo planar imaging data was acquired (flip angle = 90° , echo time = 25 ms, repetition time = 2 s) from 33 interleaved axial slices (0.5-mm gap) with a $1.8 \times 1.8 \times 3$ mm voxel size (field of view = 23 cm, 128×128 matrix).

All pre-preprocessing of the data was conducted in SPM5 (Wellcome Department of Imaging Neuroscience). Raw blood oxygen level-dependent (BOLD) data were corrected offline for slice-timing acquisition and motion artifacts. A 5-mm isotropic Gaussian smoothing kernel was applied before modeling the data. To aid in anatomical localizations of BOLD activity, we acquired high-resolution T1-MPRAGE images ($1 \times 1 \times 1$ mm voxel size, field of view = $160 \times 240 \times 256$ mm, repetition time = 2,300 ms, echo time = 3 ms, flip angle = 9°).

fMRI ROI localization. A functional localizer task was run before beginning the fMRI experiment to identify feature-selective ROIs in the extrastriate visual cortex (that is, V4 for color and V5 for motion) that are known to be modulated by attention^{29,48}. Participants were instructed to perform a one-back task in which circular apertures of color and motion stimuli (as described above) were presented in separate blocks. Each stimulus type (color and motion) was presented in 10 16-s blocks interleaved with 16 s of rest when participants passively viewed stationary gray dots. In each block, stimuli were presented for 300 ms with a 500-ms ISI. On identifying a one-back matched stimulus, participants were instructed to press the right button. There were two random matches in each color and motion block. BOLD data from the color and motion localizers were analyzed using a general linear model (GLM) and contrasted against each other. ROIs were selected in native space as the most significant cluster of activation ($P < 0.01$) in the respective anatomical region of the right hemisphere, fusiform gyrus (specifically, V4) for color and middle temporal gyrus (specifically, V5/hMT+) for motion. For all GLM analyses, epochs spanning the duration of stimulus presentation were convolved with the SPM canonical hemodynamic response function.

fMRI functional connectivity analysis. Functional connectivity network maps were created for each subject as described previously using a beta series connectivity analysis approach^{30,31}. For each condition, the encoding, maintenance and retrieval stages from every trial were modeled with their own separate regressor in the GLM and a mean beta value was extracted for each ROI (per trial). Although three stages were modeled during each trial, only the encoding period was subject to analysis. Thus, the ROI beta values from the encoding period were correlated across trials with every voxel in the brain to find regions with covariant activity. This procedure produced a whole brain Pearson's r value map for each participant and we applied a Fisher's r -to- z transformation. The z values were subsequently normalized to the Montreal Neurological Institute (MNI; $2 \times 2 \times 2$ mm voxel size) template and Gaussian smoothed (5-mm full width at half maximum) for group level analysis.

TMS. A Magstim Standard Rapid TMS Unit (Jali Medical) was used to generate pulses with a 70-mm figure-eight induction coil. The magnetic stimulus had a biphasic waveform with a pulse width of about 300 μ s. The Brainsight frameless stereotaxic software (Rogue Research) was used to co-register the subject's head, coil and high-resolution T1-weighted MRI images into a common digital workspace. The IFJ target for rTMS was identified by each individual participant's functional connectivity data (V5 seed and remember motion condition), which was subsequently overlaid onto their T1-weighted MRI image. The IFJ was targeted on the basis of fMRI connectivity data from the remember motion condition, as this condition served as a control in the rTMS experiment and the control condition results would therefore not be biased toward showing no effect. Repetitive 1-Hz TMS was applied to the right IFJ for 10 min while participants remain seated upright with the EEG cap on. Electrodes beneath the rTMS coil were removed before stimulation to minimize the distance between the coil and the head, which were replaced before EEG recording. The coil was held such that the handle protruded toward the back of the head and was approximately perpendicular to the precentral sulcus. During sham rTMS, the coil handle was held at a similar angle, but the coil face was angled 90 degrees away from the participant's head. rTMS pulse intensity was held at 65% of the maximum stimulator output for each participant. This intensity was chosen on the basis of pilot data that found it to be, on average, 120% of the active motor threshold. Following rTMS application (sham or actual), electrodes that were previously removed were replaced and participants began the task described above. Approximately 1 min elapsed between the offset of rTMS and onset of the task with EEG data collection. All participants wore earplugs during both sessions of the experiment as protection from the fMRI noise (session 1) and rTMS

clicking (session 2). The order of presentation for all conditions and application of sham/actual rTMS were counterbalanced across participants.

EEG recordings. Electrophysiological signals were recorded at 1,024 Hz through a 24-bit BioSemi ActiveTwo 64-channel Ag-AgCl active electrode EEG acquisition system (Cortech Solutions). Electrode offsets were maintained between ± 20 mV. Raw EEG data were referenced to the average off-line. Eye artifacts were removed through an independent component analysis by excluding components consistent with the electrooculogram time-series and topographies for blinks and eye movements. We extracted 1-s epochs from the data beginning 200 ms before stimulus onset and ending 800 ms after stimulus onset. This preprocessing was conducted in Brain Vision Analyzer (Cortech Solutions) and exported to Matlab (MathWorks) for all subsequent analyses. Epochs that contained an eye-related artifact were discarded from subsequent analysis. Each trial contained four stimulus epochs, two attended and two ignored, resulting in 120 epochs per block per stimulus of interest (60 per half block). Trials in which the participant responded incorrectly were discarded from analysis to ensure that neural measures reflected task-related activity. Epochs were band-pass filtered from 1–30 Hz and those that exceeded a voltage threshold of ± 50 μ V were rejected. A 200-ms pre-stimulus baseline was subtracted from each epoch before calculating the ERP. Peak P1 values were chosen as the largest amplitude between 50–150 ms and mean ERP amplitudes (± 5 ms around peak) were used for subsequent analysis. An electrode of interest was selected¹³ from the posterior right hemisphere (P4, P6, P8, P10, PO4, PO8, O2) for each subject and stimulus type (color/motion) based on the largest magnitude of modulation (attend – ignore) after averaging over the rTMS conditions (sham/actual). A repeated-measures ANOVA was implemented for ERP analysis with a Greenhouse-Geisser correction when appropriate. *Post hoc* analysis consisted of paired *t* tests. All regression analyses used Pearson's linear correlation coefficient.

EEG source localization. Sources of the P1 from the ERP were estimated using a distributed linear inverse solution. The inverse matrices were based on a local auto-regressive average (LAURA) model of the unknown current density in the

brain⁴⁹. A realistic head model was used with a solution space in the gray matter of the MNI template brain. P1 activity was averaged across subjects and over time (between 75–125 ms) and submitted to analysis through the CARTOOL software (<http://brainmapping.unige.ch/Cartool.php>). Current source density estimates were transformed to *z* scores and retained if greater than 3.

EEG phase coherence analysis. Six ROIs were identified (three frontal and three posterior). Each ROI was calculated as the average of five electrodes: F4, FC2, FC4, FC6 and C4 front-right, above the right IFJ previously identified by source localization²⁴, F3, FC1, FC3, FC5 and C3 (front-left), AFZ, FZ, F1, F2 and FCZ (front-central), P4, P6, P8, PO4 and PO8 (right-posterior), P3, P5, P7, PO3 and PO7 (left-posterior), and POZ, OZ, O1, O2 and IZ (central-posterior). Unfiltered raw epoched data from each ROI whose voltage exceeded a threshold of ± 100 μ V were rejected. Artifact free trials were then convolved with complex Morlet wavelets (family ratio: $f_o/\sigma_f = 7$) to resolve frequencies from 4 to 70 Hz. Phase was computed from the wavelet coefficients for each ROI at every time-frequency point. PLVs between two ROIs were computed by measuring the intertrial variability of the phase difference at each time-frequency point⁵⁰. This procedure yields a PLV measure bound from 0 to 1 such that 0 represents random phase differences across trials, whereas 1 indicates a consistent phase difference. PLV frequencies were averaged between 7–14 Hz (alpha band), 14–30 Hz (beta band) and 30–50 Hz (gamma band) and from –200 to 0 ms post stimulus onset to calculate pre-stimulus phase coherence. One ROI pair was selected for each frequency band for statistical analysis. Each ROI pair was selected based on the greatest attentional modulation when averaged across all conditions (alpha: front-right to central-posterior; beta: front-right to right-posterior; gamma: front-right to right-posterior).

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