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Continuous theta burst transcranial magnetic stimulation reduces resting state connectivity between visual areas

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Rahnev D, Kok P, Munneke M, Bahdo L, de Lange FP, Lau H. Continuous theta burst transcranial magnetic stimulation reduces resting state connectivity between visual areas. *J Neurophysiol* 110: 1811–1821, 2013. First published July 24, 2013; doi:10.1152/jn.00209.2013.— Continuous theta burst stimulation (cTBS) is a technique that allows for altering of brain activity. Research to date has focused on the effect of cTBS on the target area, but less is known about its effects on the resting state functional connectivity between different brain regions. We investigated this issue by applying cTBS to the occipital cortex and probing its influence in retinotopically defined regions in early visual cortex using functional MRI. We found that occipital cTBS reliably decreased the resting state functional connectivity (i.e., the correlation of spontaneous activity) between regions of the early visual cortex. In the context of a perceptual task, such an effect could mean that cTBS affects the strength of the perceptual signal, its variability, or both. We investigated this issue in a second experiment in which subjects performed a perceptual discrimination task and indicated their level of certainty on each trial. The results showed that occipital cTBS decreased both subjects' accuracy and confidence. Signal detection modeling suggested that these impairments resulted primarily from a decreased strength of the perceptual signal, with a nonsignificant trend of a decrease in signal variability. We discuss the implications of these experiments for understanding the mechanisms by which cTBS influences brain activity and perceptual processes.

cTBS; fMRI; visual cortex; perception; resting state connectivity; signal detection theory

REPETITIVE TRANSCRANIAL MAGNETIC stimulation is a popular technique used to transiently affect neural activity in a noninvasive manner. One recently developed variant of repetitive transcranial magnetic stimulation (TMS), continuous theta burst stimulation (cTBS; Huang et al. 2005), has shown promise because of its rapid application (<1 min), its ability to produce behavioral effects for up to 1 h after stimulation, and its connection to known neuronal mechanisms, such as long-term potentiation and depression (LTD). Indeed, research has shown that cTBS reduces motor cortical excitability in a manner consistent with LTD effects (Allen et al. 2007; Di Lazzaro et al. 2005, 2011; Gentner et al. 2008; Huang et al. 2005, 2007).

When applied to the occipital cortex, cTBS has been found to increase phosphene thresholds (PTs), such that higher stimulation intensity is needed to produce conscious visual experience (Franca et al. 2006). One possibility is that such an increase in PTs is, at least in part, due to decreased connectivity between areas in the early visual cortex, which would make the signal less likely to propagate to downstream areas. Such a possibility is in line with a number of recent studies that have demonstrated that the effect of TMS extends beyond the focus of stimulation, often to remote regions that are anatomically connected to the focal region (Bestmann et al. 2008; Chouinard et al. 2003; Fox et al. 2012; Garcia et al. 2011; Hampson and Hoffman 2010; Ruff et al. 2009; Zanto et al. 2011).

Here we tested for this possibility by investigating the resting state connectivity between retinotopically defined regions in the early visual cortex after occipital application of cTBS. We identified areas V1, V2, and V3 as three separate regions of interest (ROIs) and found that cTBS decreased the resting state functional connectivity between each pair of regions. In separate analyses, we separated the left and right hemisphere of each of these areas and observed that cTBS decreased the inter- and intrahemispheric resting state connectivity between these retinotopically defined regions in the primary visual cortex.

These results raised two additional questions: *1*) is the decreased resting state functional connectivity related to behaviorally relevant effects, and, if so, *2*) would cTBS have a larger effect on the strength or on the variability of the perceptual signal. We addressed these questions in a second experiment in which subjects performed a stimulus discrimination task before and after receiving theta burst stimulation. We found that occipital cTBS produced decreases in performance and confidence. To address whether this effect was due to a decrease in signal strength, increase in variability, or both, we fitted the behavioral data to a signal detection theoretic model informed by our laboratory's previous work (Rahnev et al. 2011, 2012). The main idea of the modeling was that, by assuming constant decision criteria between conditions, one could analytically separate the effects of signal strength and signal variability. To anticipate, our results suggested that cTBS decreased the strength of the perceptual signal without significantly affecting its variability.

METHODS

Subjects. Five subjects participated in the first experiment (*experiment 1*: cTBS-fMRI), in which functional MRI (fMRI) activity was recorded before and after applying cTBS. A technical problem led to losing the fMRI data from one subject in *experiment 1*. The other four subjects in *experiment 1* were authors M. Munneke, P. Kok, F. de Lange, and L. Bahdo (2 women, 23–32 yr old). Even though they were not naive to the purpose of the study, this part of the experiment required no active participation in any psychophysical tasks.

Thirteen naive subjects participated in the second experiment (*experiment 2*: cTBS-behavior), where we compared subjects' performance on a psychophysical task before and after applying cTBS. Three subjects did not perceive phosphenes with occipital TMS and

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were excluded from the analyses. One other subject dropped out after the first three (out of four) sessions of the experiment and was also excluded from the analyses. Thus the final analysis included data from nine subjects (6 women, 19–26 yr old).

Subjects from both experiments had normal or corrected-to-normal vision. They received detailed information about the potential side effects of cTBS. A written, informed consent was obtained from all subjects. The research was approved by the local ethics committee in which the experiment was performed (CMO region Arnhem-Nijmegen, The Netherlands).

PT determination. For both experiments, we determined the PT on the first day of the respective experiment using a method similar to Rahnev et al. (2012). Briefly, we first used a "hunting procedure" to determine the optimal location for stimulation on the occipital cortex. Specifically, we applied suprathreshold single pulses of TMS to find a location on the back of the head that produced a clear phosphene at the center of the visual field. This allowed us to stimulate the occipital cortex without targeting a specific quadrant of retinotopically defined visual cortex in *experiment 1*, and also to present the visual stimuli in the same location for all subjects in *experiment 2*. Following previous research (Boyer et al. 2005), we chose the starting point of the hunting procedure to be 2 cm above and 1 cm left of the inion, but the final position was close to the midline for all subjects, and thus it is unlikely that one hemisphere was targeted preferentially. We could not estimate the induced electrical field in each retinotopically defined visual area as has been done before (Kammer et al. 2001; Salminen-Vaparanta et al. 2012a, 2012b; Thielscher et al. 2010), but instead focused on the functional consequences of stimulation rather than in the precise amount of stimulation induced in each region.

We then proceeded to determine each individual's PT using procedures similar to what is commonly done for determining motor thresholds (Rossini et al. 1994; Rothwell et al. 1999; see also Abrahamyan et al. 2011). More specifically, starting at 30% of the maximum stimulator output, we delivered single-pulse TMS until we reached the lowest intensity at which a subject reported perceiving phosphenes on 5 out of 10 trials; this intensity was chosen as the subject's PT. Throughout this procedure and subsequent application of cTBS, the main axis of the coil was oriented parallel to the sagittal plane, and the coil handle extended ventrally as in our previous work (Rahnev et al. 2012).

Theta burst stimulation. For both *experiments 1* and *2*, cTBS was delivered with a Magstim Super Rapid Stimulator (Magstim, Whitland, UK) connected to four booster modules, using a figure-of-eight coil with a diameter of 70 mm. The stimulation lasted 40 s during which we delivered five bursts of three 50-Hz pulses every second for a total of 600 pulses (Huang et al. 2005). The stimulation was delivered at 80% of the individual PT. The intensity of TMS to the control sites (vertex in *experiment 1* and Pz in *experiment 2*) was always the same as for the occipital cortex. To ensure exactly the same stimulation intensity across sessions, we did not remeasure the PT beyond *day 1* of each experiment (see below). This likely introduced some variability in the stimulation intensity compared with the true PT, because PT is likely to change from day to day. Thus it is likely that, even though we aimed to stimulate at exactly 80% of PT, the actual intensity was 80% of PT in the group, but varied somewhat in individual subjects. No leg or other movement was elicited by vertex or Pz stimulation in any of the subjects.

Experiment 1: procedure. Experiment 1 investigated whether resting state functional connectivity between areas in the early visual cortex is altered by offline cTBS. The experiment took place over 3 separate days. On *day 1*, we determined the PT of the subject as described above. On *days 2* and *3*, we collected functional magnetic resonance imagining data of subjects' occipital cortex before and after they received cTBS. We stimulated the occipital cortex on 1 of the days and the vertex of the head on the other day. The vertex was used as a control site, and the order of stimulation sites for *days 2* and *3* was counterbalanced between the subjects. The mean intensity of stimulation was 54.5% (SD = 12.8%; the individual intensities were 44, 44, 60, and 70%) of maximum stimulator output.

On both *days 2* and *3*, subjects arrived at the site, were screened for metallic objects, and were positioned in the MRI scanner. We then collected a retinotopy session using a rotating wedge for 8 min (314 volumes), then a resting state session for 10 min (399 volumes), and then another identical rotating-wedge retinotopy session. Subjects were then escorted out of the scanner to an adjacent room with the TMS equipment. In the vertex condition, we simply stimulated the site at the predetermined intensity. In the occipital condition, we performed the same hunting procedure (see above) to find the optimal stimulation site and then applied cTBS at the same predetermined intensity. During the stimulation, subjects were given a blindfold that they kept on until they were placed back in the scanner. After the stimulation, subjects were escorted back to the scanner as quickly as possible such that scanning resumed approximately 5 min after the stimulation. The same sequence of three scanning sessions (retinotopy-resting state session-retinotopy) was performed again, in the same order as the pre-cTBS session.

In addition, for each subject, on either *day 2* or *3*, we administered an additional retinotopy session in which an annulus expanded and contracted for 10 min (410 volumes). This retinotopy session was distinct from the rotating wedge sessions described above and was used to map visual eccentricity (see *Experiment 1*: *retinotopy* below). Furthermore, we also collected an anatomical image of the visual cortex with the same coil as the one used to collect the functional data and a whole brain anatomical image with a separate full-head coil. These additional scanning sessions were performed after the completion of the main scanning session after all of the sessions described above were already collected.

Experiment 1: fMRI acquisition. Images were acquired on a 3 Tesla Trio MRI system (Siemens). Functional images were acquired using an 8-channel occipital coil, with a single-shot gradient echo-planar imaging sequence (repetition time, 1,500 ms; echo time, 30 ms; 23 ascending slices; voxel size, $2 \times 2 \times 2$ mm; flip angle, 70°; field of view, 220 mm). A high-resolution anatomical image was acquired using a T1-weighted MPRAGE sequence (repetition time, 2,300 ms; echo time, 3.52 ms; voxel size, $0.8 \times 0.8 \times 0.8$ mm) with the same field of view as the functional images. In addition, another high-resolution anatomical image was acquired with a 32-channel coil using a T1-weighted MPRAGE sequence (repetition time, 2,300 ms; echo time, 3.03 ms; voxel size, $1 \times 1 \times 1$ mm), with the whole brain in field of view.

Experiment 1: retinotopy. The boundaries of retinotopically defined areas in early visual cortex were identified using traveling-wave methods (Engel et al. 1997; Sereno et al. 1995). Visual field positions can be expressed as polar coordinates, i.e., in terms of angle and eccentricity. Angle was mapped by having subjects view a wedge, consisting of a flashing checkerboard pattern (3 Hz), first rotating clockwise for 9 cycles and then counterclockwise for another 9 cycles (at a rotation speed of 18 s/cycle). In a similar vein, eccentricity was mapped by presenting subjects with expanding (9 cycles, 18 s/cycle) and contracting (9 cycles) rings of flashing checkerboard patterns (3 Hz), centered on fixation. Fourier-based methods were used to obtain both the amplitude and the phase of the blood-oxygenation-level-dependent signal at the fundamental frequency of the stimuli (1/18 Hz). While the amplitude of the signal at this frequency, relative to the signal at other frequencies, can be seen as an indication of the signal-to-noise ratio, the phase can be used to construct polar angle and eccentricity maps of the cortical surface. The borders of the visual areas (dorsal and ventral V1, V2, and V3) were defined on the basis of these maps, using Freesurfer (http://surfer.nmr.mgh.harvard.edu/). These retinotopic maps were then used to create ROIs using MarsBaR (http://marsbar.sourceforge.net/).

Experiment 1: analyses. Once we identified the ROIs corresponding to different brain regions, we extracted time courses for each of them from the resting state sessions. For the main analysis, we combined the differ-

ent subregions (i.e., dorsal and ventral, and left and right hemisphere) of V1, V2, and V3 so that we could look at the time course correlations for these regions as a whole. Besides that, we also performed planned tests between left and right V1, left and right V2, and left and right V3. Additional exploratory analyses looked at the intrahemispheric connectivity between the left hemisphere V1, V2, and V3, and separately between the right hemisphere V1, V2, and V3.

For each analysis, we discarded the first six volumes to allow for scanner equilibration. The time course of all cerebral spinal fluid voxels was regressed out to control for nonneural influences. Each time course was then normalized to have a mean of zero and a standard deviation of one. Finally, we correlated the time courses of different pairs of regions. Since the correlation coefficient r is not on a linear scale, we transformed each r value using the Fisher r-to-Z transformation (equivalent to the inverse hyperbolic tangent; Fisher 1915):

$$r_z = \tfrac{1}{2} * \left[\ln(1 + r) - \ln(1 - r)\right] \quad (1)$$

For each pair of regions (i.e., V1 and V2, or left and right V1), we obtained four different transformed correlation coefficients: pre-cTBS to occipital cortex ($r_{z\_pre\_occipital}$), post-cTBS to occipital cortex ($r_{z\_post\_occipital}$), pre-cTBS to vertex ($r_{z\_pre\_vertex}$), and post-cTBS to vertex ($r_{z\_post\_vertex}$). Based on the first two values, we computed the effect of occipital cTBS, and based on the last two values, we computed the effect of vertex cTBS. To do so, we applied the following formula introduced by Fisher (1921):

$$r_{diff} = (r_{z\_post} - r_{z\_pre}) / \mathrm{sqrt}\left[1/(n_{post} - 3) + 1/(n_{pre} - 3)\right] \quad (2)$$

where n_{post} and n_{pre} signify the number of data points from which the r_{z_post} and r_{z_pre} correlations were computed, respectively. The value of n was calculated based on the effective sample size in the following manner. Each time course consisted of 393 volumes, since we had collected 399 volumes in all resting state sessions, but had discarded the first 6 volumes to allow for scanner equilibration (see above). However, due to the presence of autocorrelation, the effective degrees of freedom in each time course were lower. To estimate the effective degrees of freedom (Santer et al. 2000, 2008), we used the derivation by Priestley (1981) and used the formula:

$$n_{effective} = n_{total} * (1 - r_{auto}) / (1 + r_{auto}) \quad (3)$$

where r_{auto} is the amount of autocorrelation present in the data. To estimate the true value of r_{auto}, for each session of each subject, we computed the autocorrelation of each retinotopically defined region and took a geometric average of these values as an estimate of the true autocorrelation for that subject in that session. Other ways of estimating r_{auto}, such as averaging across sessions, or even further averaging across subjects did not change the results. The value of $n_{effective}$ was used for estimating r_{diff} in *Eq. 2*.

The variable r_{diff} obtained in *Eq. 2* has a standard normal distribution N(0,1) (Fisher 1921), i.e., a Gaussian distribution with mean 0 and standard deviation 1 (which is also equivalent to a z-score). Therefore, we could use the r_{diff} values computed for occipital cTBS ($r_{diff\_occipital}$) and vertex cTBS (r_{diff_vertex}) to directly compare the effects of occipital and vertex cTBS. Since $r_{diff\_occipital}$ and r_{diff_vertex} are mathematically independent and have N(0,1) distributions, $r_{diff\_occipital} - r_{diff\_vertex}$ has N[0,sqrt(2)] distribution. Therefore,

$$r_{effect} = (r_{diff\_occipital} - r_{diff\_vertex}) / \mathrm{sqrt}(2) \quad (4)$$

has a standard normal N(0,1) distribution. We computed r_{effect} for each subject and used it to determine significance by transforming the z-score into a P value. We report both the z-score and P value for all tests.

Since we only had four subjects, it was impossible to compute a random-effect group statistic. We therefore computed a fixed-effect statistic in the following manner. Since the r_{effect} values above have N(0,1) distribution, we summed these values for the four subjects which produced a variable with N(0,2) distribution. We therefore divided the sum of the four r_{effect} variables by 2 to obtain an N(0,1)-distributed variable that we could use to determine significance. It should be noted that this group test is a "fixed-effect" statistic, meaning that the effects uncovered are only valid when applied to our subjects, but cannot be used to generalize beyond them to other subjects from the general population. We report this statistic for completeness, but note that any inferences are more appropriately made based on the pattern of individual results rather than the fixed-effect statistic. As above, we report both the z-score and P value for all group fixed-effect tests.

Experiment 2: theta burst stimulation. Experiment 2 investigated whether cTBS would produce behaviorally relevant aftereffects in a psychophysical task. Since cTBS reduces cortical excitability (Huang et al. 2007), one may predict that it will lead to a reduction in the strength of the perceptual signal. Nevertheless, at least one study has observed performance improvements after cTBS to the occipital cortex (Waterston and Pack 2010), so we also entertained the possibility that cTBS could lead to an increase in signal variability.

The experiment took place over 4 different days. On *day 1*, we determined subjects' PTs as described above, and then subjects practiced performing the psychophysical task (see below). *Days 2–4* involved theta burst stimulation to the occipital cortex, Pz (parietal midline; standard EEG nomenclature), and sham stimulation. As in *experiment 1*, in the occipital stimulation session, we determined the correct stimulation site by running an additional hunting procedure on the day of occipital theta burst stimulation. For both occipital and Pz stimulation, we used 80% of the originally determined PT (46.6%, SD = 18.9% of maximum stimulator output). For the sham stimulation, we used 15% of the maximum stimulator output and placed the coil on top of the head, but positioned it perpendicularly to the scalp so that only the side of the coil touched the head (and therefore only negligible amount of the magnetic field could reach the skull). We used both low stimulator intensity and tilting of the coil because we were concerned about any induced electric field reaching the cortex. As it turned out, the Pz and sham stimulation conditions did not differ from each other (see RESULTS), and thus it is unlikely that subjects treated the sham condition any differently than the Pz or occipital stimulation conditions. The two active cTBS sessions (occipital and Pz) were performed at least 1 wk apart from each other.

Experiment 2: task. Subjects' task was to indicate the tilt (clockwise or counterclockwise) of a grating presented at fixation: the same location in which phosphenes were induced. Each trial began with 50-ms presentation of the grating followed by a fixation period of 200 ms (see Fig. 3). On each trial the orientation of the grating was randomly selected to be tilted 10° clockwise or 10° counterclockwise away from vertical. The grating pattern was presented on an annulus (inner circle radius: 1.5°, outer circle radius: 4.5°) region. The grating stimulus consisted of a noisy background composed of uniformly distributed intensity values (8% contrast) on top of which we added a grating (0.5 cycles/°). Subjects were required to fixate on a small white square for the duration of the experiment. They were seated in a dim room 50 cm away from a computer monitor. Stimuli were generated using Psychophysics Toolbox (Brainard 1997) in MATLAB (MathWorks, Natick, MA) and were shown on a MacBook (13-in. monitor size, 1,200 × 800 pixel resolution, 60-Hz refresh rate).

After each stimulus presentation, subjects used one of four keys to give their response indicating the perceived orientation of the grating and a wager on whether they were correct. Subjects used the *keys 1–4* indicating "certainly left," "guess left," "guess right," and "certainly right," respectively. A correct "certain" (i.e., high confidence) choice was awarded with two points, while a correct "guess" (i.e., low confidence) choice was awarded with one point. An incorrect "guess" (i.e., low confidence) choice resulted in no points being won or lost, but an incorrect "certain" (i.e., high confidence) choice resulted in a loss of two points. We chose this point structure to ensure that subjects gave a sufficient number of both "guess" and "certain" responses. The

optimal strategy for this payoff structure was to choose the "certain" choice only when the probability of being correct exceeded 66.7%. We informed subjects of this contingency to guarantee that all subjects were aware of the optimal strategy. To further encourage optimal usage of the wagers, we gave the two subjects with highest final scores an additional cash prize. Since the wagers that subjects used were a proxy for their confidence on each trial, for simplicity we refer to the wagers as confidence ratings in the rest of the paper.

Each trial lasted for 2 s. Subjects had 1.8 s to give their response after the onset of the stimulus. Once a response was given, the text indicating the four possible answers (see Fig. 3) disappeared and the next trial started. If a response was not given in the 1.8-s period, subjects were penalized by a subtraction of four points, and the text was removed at the end of the 1.8-s period to avoid any potential interference with the processing of the stimulus in the next trial.

Experiment 2: session sequence. In the initial training session on *day 1*, subjects practiced with the task over the course of five blocks of 120 trials each. In the first three blocks, subjects received trial-by-trial feedback to learn to give confidence ratings as optimally as possible. The last two blocks did not involve trial-by-trial feedback to prepare subjects for the actual experiment (*days 2–4*). In the blocks for which trial-by-trial feedback was provided, each trial was extended to 2.5 s in order for subjects to be able to clearly see the feedback.

Days 2–4 involved theta burst stimulation to the occipital cortex, Pz, and sham cTBS (in a counterbalanced order between subjects). Based on the results of the training session on *day 1*, we chose a grating contrast for each subject that would produce ~80% correct responses. However, since some subjects tend to be biased and use conservative or liberal wagering strategies (Dienes and Seth 2010), we decided to include two more levels of contrast: 75 and 125% of the above contrast. These three contrast levels were used on *days 2–4* without further adjustments, even if performance deviated from the 80% correct target for the middle contrast. Contrast level was chosen randomly on each trial, and subjects were not explicitly informed about the presence of multiple contrast levels. Since contrast was not a variable of interest and was only used in order for subjects to use sufficiently both levels of confidence, for the purposes of the analyses we averaged across the three levels of contrast.

To provide further training with the task, each of the three cTBS sessions on *days 2–4* started with 40 training trials that were not analyzed. These trials were presented only in the beginning of the session before magnetic stimulation and were clearly marked as training trials. Then subjects completed five blocks of 140 trials each. Each block lasted 280 s (4 min and 40 s) and was followed by 20 s of rest for a total duration of 5 min per block. During the 20-s break after each block subjects were given feedback on their accumulated score from the last block, but no trial-to-trial feedback was provided.

Experiment 2: data analysis. The signal detection theoretic measure d′ that quantifies subjects' sensitivity was calculated by first coding each trial as a hit, miss, false alarm, or a correct rejection. Trials in which subjects reported that the stimulus was tilted in clockwise direction were coded as hits, if the grating was indeed tilted clockwise, and as false alarms otherwise. Trials in which subjects reported that the stimulus was tilted in counterclockwise direction were coded as correct rejections, if the grating was indeed tilted in counterclockwise direction, and as misses otherwise. Hit rate (HR) was computed as hits/(hits + misses), and false alarm rate (FAR) was computed as false alarms/(false alarms + correct rejections). Finally, d′ was calculated as:

$$\mathrm{d'} = z(\mathrm{HR}) - z(\mathrm{FAR})$$

where z is the inverse of the cumulative standard normal distribution that transforms HR and FAR into z-scores.

The question that we were trying to test was whether occipital cTBS differed from Pz and sham stimulation. To investigate this question, we first checked for differences between the Pz and sham stimulation conditions. We found that they did not differ in any systematic way (see RESULTS section), and therefore we combined them as "control" conditions to be compared with occipital stimulation. We chose this approach over running an ANOVA with three factors (each stimulation site), since that would have tested for differences between any two conditions, which was not the focus of our study, and decreased our power to detect differences between occipital stimulation and the two control conditions.

The main analysis sought to determine whether cTBS had an aftereffect on d′ and confidence level. We compared the pre-cTBS scores and the post-cTBS scores for each subject by computing the percent change of the post-cTBS scores as a function of the pre-cTBS scores. Then we compared these percent change values for the occipital and control conditions using paired t-tests. As a control analysis, we further performed a 2×2 repeated-measures ANOVA with factors time of test (pre-cTBS or post-cTBS) and condition (occipital or control stimulation). The interaction of the two factors represented the effect of occipital cTBS that we were interested in. The results of this control analysis mirrored the main analysis described above.

Experiment 2: signal detection modeling. We used signal detection theory (SDT) analyses to uncover parameters related to the separation and variance of the internal distributions. We refer to these parameters as "signal strength" and "signal variability" in the rest of the paper. Behaviorally, we observed a decrease in d′ after cTBS to the occipital cortex. We reasoned that this effect could be due to signal decrease, variability increase, or both. Nevertheless, it is also possible that the ratio of the variability and the mean (known as Fano factor) would remain relatively constant. Indeed, such effect has been observed for single neurons (e.g., Dean 1981). This line of reasoning would predict that signal strength and signal variability would be affected in a similar way by cTBS (both increasing or decreasing), but that the effects would have a different magnitude so that a difference in d′ emerges.

To perform the SDT modeling, we made some standard assumptions: *1*) the two stimuli used (clockwise and counterclockwise gratings) give rise to Gaussian distributions on some decision axis; *2*) the discrimination decision is made by comparing the signal on the decision axis with a criterion, while the confidence judgments are made by comparing the signal on the decision axis with two flanking criteria, such that "certain" judgments are only produced in the two extremes of the decision axis; and *3*) the criteria for perceptual decisions and confidence judgments are set in the same way for each day of the experiment (that is, they are the same for the pre-TMS and post-TMS sessions on each day). The last assumption is justified by our use of a wagering procedure that encourages constant placement of decision criteria, as well as by previous research that demonstrated that subjects used unified decision criteria for different conditions within an experiment, even when such unified criteria were clearly suboptimal (Gorea and Sagi 2000).

For both occipital and control conditions, we were interested in the distance μ between the peaks of the Gaussian distributions and the standard deviation σ of these distributions. We modeled these separately for the pre-TMS and post-TMS sessions for both the occipital and control TBS. Therefore, we initially had eight parameters related to the signal strength and signal variability: $\mu_{\mathrm{pre\_occ}}$, $\mu_{\mathrm{pre\_control}}$, $\mu_{\mathrm{post\_occ}}$, $\mu_{\mathrm{post\_control}}$, $\sigma_{\mathrm{pre\_occ}}$, $\sigma_{\mathrm{pre\_control}}$, $\sigma_{\mathrm{post\_occ}}$, and $\sigma_{\mathrm{post\_control}}$. To achieve a unique solution for these parameters, we set $\sigma_{\mathrm{pre\_occ}}$ and $\sigma_{\mathrm{pre\_control}}$ to an arbitrary constant value of 1. The resulting model had 12 free parameters: $\mu_{\mathrm{pre\_occ}}$, $\mu_{\mathrm{pre\_control}}$, $\mu_{\mathrm{post\_occ}}$, $\mu_{\mathrm{post\_control}}$, $\sigma_{\mathrm{post\_occ}}$, $\sigma_{\mathrm{post\_control}}$, and the location of each of the three criteria levels used for discrimination and confidence judgments for the occipital stimulation (3 parameters) and control stimulation (3 parameters) sessions. Our model had a large number of parameters, and thus what is interesting here is not whether or not it would be able to fit the data, but rather whether or not cTBS would have a consistent aftereffect on μ and σ. One of the subjects had very high percentage of

high-confidence responses (up to 97% in some conditions). This made the estimated values of μ and σ highly variable, and therefore that subject was excluded from this analysis.

We fit the models to the data as done previously (Rahnev et al. 2011, 2012) using a maximum likelihood estimation approach that has previously been used within a signal detection framework (Dorfman and Alf 1969). The models were fit to the full distribution of probabilities of each response type contingent on each stimulus type. The model fitting was done by finding the maximum-likelihood parameter values using a simulated annealing procedure (Kirkpatrick et al. 1983). Model fitting was conducted separately for each subject's data.

RESULTS

Experiment 1: cTBS decreases resting state functional connectivity. We investigated whether occipital cTBS led to a significant change in the resting state functional connectivity between regions in the early visual cortex during a resting state session. To this end we defined V1, V2, and V3 (as well as their subregions) in each subject (Fig. 1) and computed the resting state connectivity for each pair of regions for each subject.

The results showed that, compared with control cTBS, occipital cTBS led to a significant decrease in resting state functional connectivity between V1 and V2 on the group level ($z = 4.8$, $P < 0.0001$; Fig. 2*A*). The same was true about the resting state connectivity between V1 and V3 ($z = 5.11$, $P < 0.00001$), as well as between V2 and V3 ($z = 4.66$, $P < 0.0001$). These group level analyses represent fixed-effect statistics; therefore, any inferences about the reliability of the results outside of our group of subjects are best made based on the pattern of the individual data.

The individual data suggest that these results could be seen in most subjects, although there was a certain amount of variability. Specifically, the resting state connectivity between V1 and V2 was significantly affected by cTBS in three out of the four subjects, with the last one showing a trend in the same direction (S1: $z = 1.89$, $P = 0.059$; S2: $z = 2.47$, $P = 0.013$; S3: $z = 2.99$, $P = 0.003$; S4: $z = 2.24$, $P = 0.025$). The same pattern involving three significant results, and one trend was observed for the resting state connectivity between V1 and V3 (S1: $z = 2.18$, $P = 0.029$; S2: $z = 2.94$, $P = 0.003$; S3: $z = 3.31$, $P = 0.0009$; S4: $z = 1.78$, $P = 0.074$). The individual data were less consistent for the resting state connectivity between V2 and V3 with only two subjects showing significant effects (S1: $z = 4.74$, $P < 0.0001$; S2: $z = 1.64$, $P = 0.102$; S3: $z = 0.48$, $P = 0.635$; S4: $z = 2.47$, $P = 0.013$).

We also checked the aftereffect of occipital cTBS on the resting state functional connectivity between the left and right parts of V1, V2, and V3 (Fig. 2*B*). Similar to the above results, we found that, compared with control cTBS, occipital cTBS significantly decreased the resting state connectivity between left and right V1 ($z = 6.25$, $P < 0.0001$), left and right V2 ($z = 3.8$, $P = 0.0001$), and left and right V3 ($z = 3.63$, $P = 0.0003$). Again, the individual data showed the same pattern but were less consistent. Specifically, the resting state connectivity between left and right V1 was affected by cTBS for two

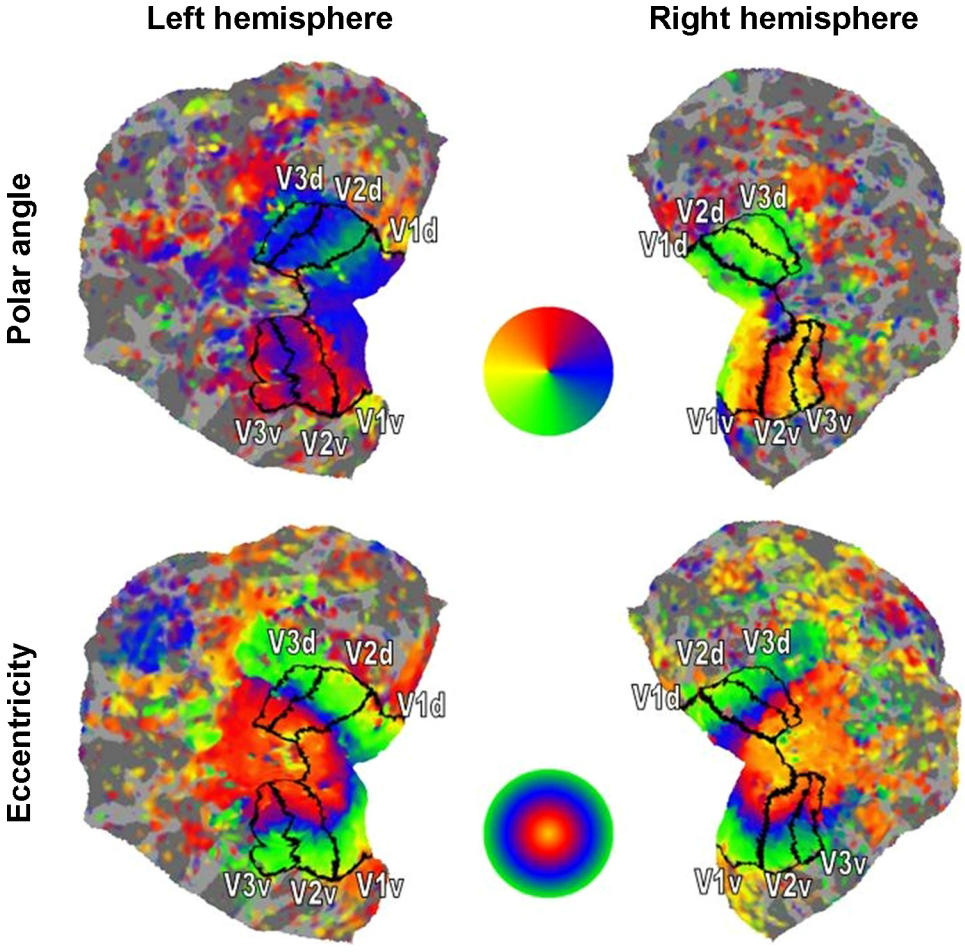

Fig. 1. Retinotopically defined regions in early visual cortex. The maps for polar angle (*upper* part) and eccentricity (*lower* part) are shown for both the left hemisphere (*left* part) and right hemisphere (*right* part) for one subject. The maps are overlaid on the flattened occipital cortex of that subject. Black lines represent borders of the early visual areas V1, V2, and V3. v = ventral, d = dorsal.

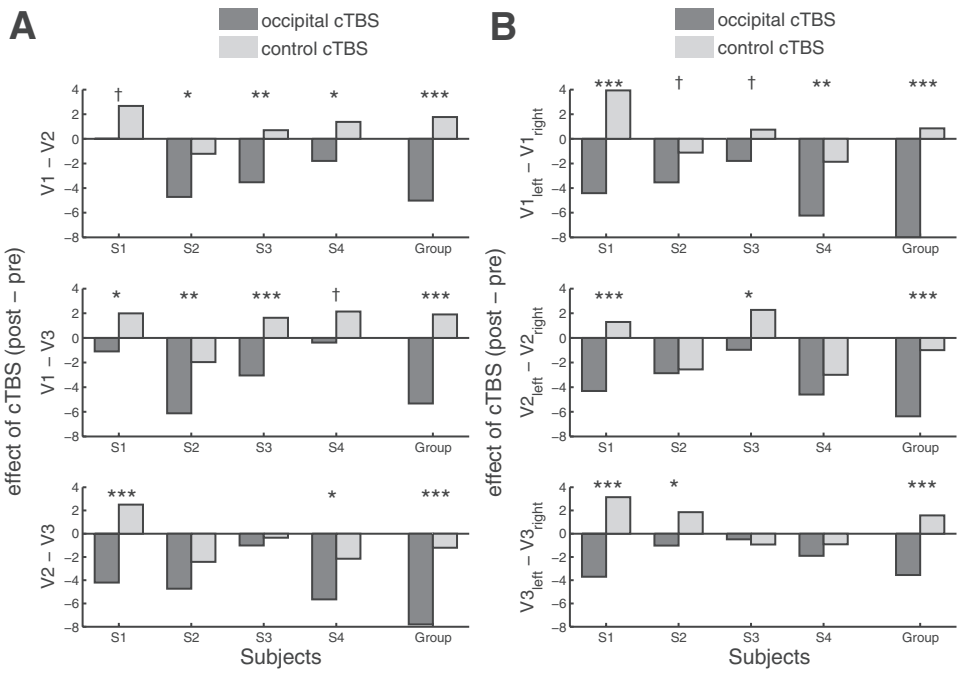

Fig. 2. Effect of continuous theta burst stimulation (cTBS) on the resting state functional connectivity between regions in the early visual cortex. *A*: compared with control (vertex) theta burst stimulation, occipital cTBS led to a decrease in resting state functional connectivity between V1 and V2, i.e., the difference in the strength of the correlation before and after cTBS was greater for occipital than for control cTBS. The same was true for the resting state connectivity between V1 and V3, as well as between V2 and V3. The figure presents the individual results of each subject, as well as a fixed-effects group result. *B*: compared with control stimulation, occipital cTBS also led to a decrease in resting state connectivity between left and right V1. The same effect was true for both V2 and V3. All data in the figure are z-scores. †$P < 0.1$, *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$.

out of the four subjects, with the other two subjects showing trends in the same direction (S1: $z = 5.9$, $P < 0.0001$; S2: $z = 1.71$, $P = 0.087$; S3: $z = 1.8$, $P = 0.072$; S4: $z = 3.09$, $P = 0.002$). The same analysis revealed significant effects for two subjects for both V2 (S1: $z = 3.96$, $P < 0.0001$; S2: $z = 0.22$, $P = 0.828$; S3: $z = 2.29$, $P = 0.022$; S4: $z = 1.13$, $P = 0.257$) and V3 (S1: $z = 4.84$, $P < 0.0001$; S2: $z = 2.04$, $P = 0.042$; S3: $z = -0.32$, $P = 0.75$; S4: $z = 0.7$, $P = 0.48$). Therefore, it appears that differences in individual anatomy brought about significant variability in the pattern of individual results though the effects emerged consistently on the group level.

Furthermore, we checked whether these effects were stronger in either the left or right hemispheres. We computed the pairwise correlations between left hemisphere V1, V2, and V3, as well as between the right hemisphere V1, V2, and V3. On the group level, we found that cTBS to occipital cortex decreased resting state functional connectivity for all six pairs of regions (all z-scores > 3.5, all P values < 0.0005). Overall, out of the 12 single-subject tests in the left hemisphere, 7 showed a significant effect, and 1 showed a trend (left V1/V2: S1: $z = 2.02$, $P = 0.043$; S2: $z = 4.38$, $P < 0.0001$; S3: $z = 3.45$, $P = 0.0006$; S4: $z = 4.67$, $P < 0.0001$; left V1/V3: S1: $z = 1.33$, $P = 0.18$; S2: $z = 2.86$, $P = 0.004$; S3: $z = 1.93$, $P = 0.054$; S4: $z = 0.89$, $P = 0.38$; left V2/V3: S1: $z = 4.33$, $P < 0.0001$; S2: $z = 6.27$, $P < 0.0001$; S3: $z = 0.79$, $P = 0.43$; S4: $z = 1.22$, $P = 0.22$). The effects were very similar in the right hemisphere where seven single-subject tests were significant (right V1/V2: S1: $z = 5.98$, $P < 0.0001$; S2: $z = 0.33$, $P = 0.74$; S3: $z = 2.48$, $P = 0.013$; S4: $z = 0.98$, $P = 0.33$; right V1/V3: S1: $z = 5.23$, $P < 0.0001$; S2: $z = 0.28$, $P = 0.78$; S3: $z = 3.38$, $P = 0.0007$; S4: $z = 3.6$, $P = 0.0003$; right V2/V3: S1: $z = 5.46$, $P < 0.0001$; S2: $z = -0.22$, $P = 0.82$; S3: $z = 0.11$, $P = 0.91$; S4: $z = 1.97$, $P = 0.049$). It is also important to note that three out of the four subjects showed at least one significant effect in both hemispheres. Thus it appears that the decreased resting state connectivity was not localized to a specific pair of regions or to a specific hemisphere.

Experiment 2: modeling rationale. Experiment 1 showed that occipital cTBS caused a decrease in resting state functional connectivity across the visual cortex. This result raised two additional questions: *1*) is the decreased resting state functional connectivity related to behaviorally relevant outcomes, and, if so, *2*) would cTBS have a larger aftereffect on the strength of the perceptual signal or its variability. We addressed these questions in *experiment 2* in which, before and after theta burst stimulation, subjects completed a stimulus discrimination task and gave confidence ratings on each trial.

To address our second question, the behavioral data were fit with a SDT model inspired by our laboratory's previous work (Rahnev et al. 2011, 2012). The main idea of the modeling was that, if constant decision criteria between conditions are assumed (Gorea and Sagi 2000), then one expects different effects of decreased signal strength and increased variability.

Figure 3*B* depicts visually a situation where a subject discriminates between two stimulus alternatives (i.e., S1 vs. S2). The decision about stimulus identity is performed using a single discrimination criterion that is usually set close to the middle between the peaks of the two distributions. On the other hand, confidence ratings are placed using additional confidence criteria, such that high confidence is given only when the signal is extreme in either direction (see *upper* panel of Fig. 3*B*). The SDT measure of performance sensitivity (d′) is computed as the distance between the peaks of the distributions divided by their standard deviation.

A decrease in signal strength (see dashed distributions in the *middle* panel of Fig. 3*B*) results in lower d′ because the peaks of the distributions move closer together, but the standard deviations do not change. Such signal decrease is always accompanied by decreased confidence (note that the dashed distributions in the *middle* panel of Fig. 3*B* extend less into the high-confidence regions). On the other hand, an increase in signal variability (see dashed distributions in *lower* panel of Fig. 3*B*) also results in lower d′, because the distance between the peaks of the distributions remains the same, but the stan-

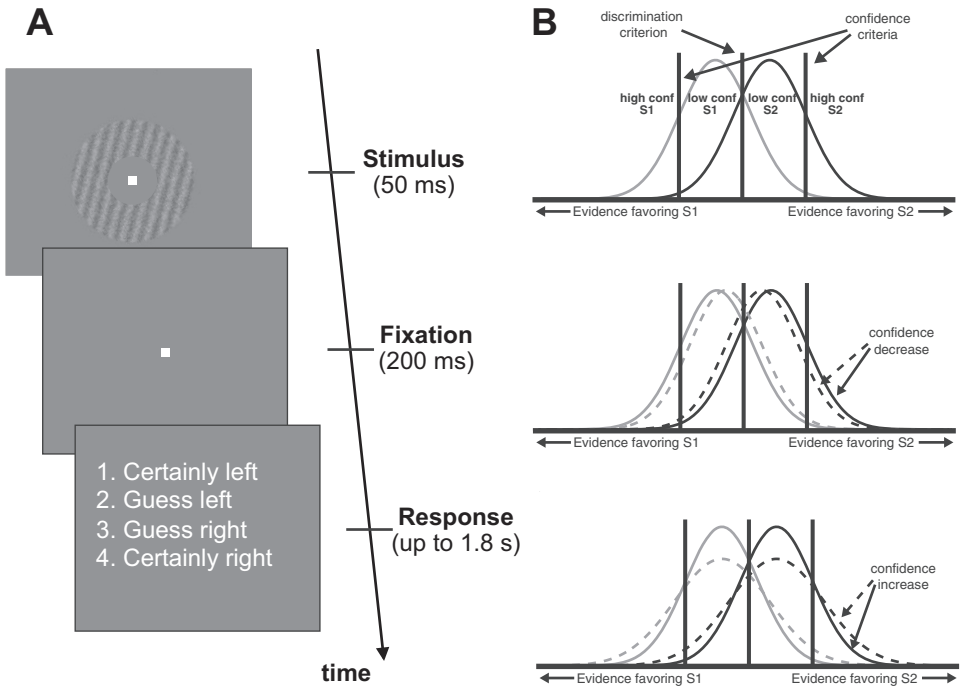

Fig. 3. *Experiment 2*: task design and signal strength vs. signal variability analysis. *A*: subjects discriminated whether a grating was tilted 10° to the right (clockwise) or left (counterclockwise) from vertical. Subjects also wagered on their level of certainty such that a high-confidence response earned them more points when they were correct but led to loses when they were wrong (see METHODS for details). Each trial lasted exactly 2 s, and missed responses were punished with point losses. *B*: According to signal detection theory (SDT), subjects distinguish between two stimulus alternatives (i.e., S1 vs. S2) by adopting a discrimination criterion, while confidence is given with the use of additional "confidence criteria" (see *upper* panel). If a manipulation decreases the signal strength (dashed distributions, *middle* panel), this is accompanied by decreased confidence (the dashed distributions in the panel extend less into the high-confidence regions). On the other hand, if a manipulation increases the signal variability (dashed distributions, *lower* panel), this may be accompanied by increased confidence (the dashed distributions in the panel extend more into the high-confidence regions). These effects depend on the exact placement of the confidence criteria. For both the *middle* and *lower* panels, the dashed distributions represent the same level of d′ (signal detection theoretic measure of sensitivity) decrease (~25%).

dard deviations increase. In fact, the *middle* and *lower* panels of Fig. 3*B* depict situations where d′ decreases about the same amount (by 25%). Interestingly, however, the variability increase is normally accompanied by increased confidence (the dashed distributions in the *lower* panel of Fig. 3*B* extend more into the high-confidence regions). This effect on confidence ratings depends on the exact placement of the confidence criteria; in fact, if the confidence criteria were placed close to the discrimination criterion such that only few low-confidence responses are produced, then an increase in signal variability would also lead to decreased confidence. Nevertheless, even in that situation, the decrease in confidence caused by an increase in the signal variability would be lower than that caused by a decrease in signal strength. One implication is that the influence of cTBS on signal strength and signal variability cannot be determined by looking at the mean confidence alone, but is a computationally tractable problem using formal SDT modeling.

Experiment 2: cTBS impairs the perceptual signal. We applied the logic outlined above to the data from *experiment 2*. Behaviorally, we hypothesized that cTBS to the occipital cortex would lead to a decrease in d′ compared with cTBS to Pz (a control site) or sham cTBS. We first checked for any differences between Pz cTBS and sham cTBS. We compared the "effect of cTBS" for each of these two types of stimulation by computing the percent change from subjects' scores on different measures before cTBS administration (pre) to the scores after cTBS administration (post). Paired-sample *t*-tests on these change scores for Pz and sham stimulation revealed no influence of type of stimulation on subjects' d′ [$t(8) = 0.59$, $P = 0.57$], confidence ratings [$t(8) = -0.43$, $P = 0.68$], overall points earned [$t(8) = -0.15$, $P = 0.88$], or reaction times [$t(8) = 0.4$, $P = 0.7$]. We therefore averaged the Pz and sham cTBS sessions as "control cTBS" and compared that to occipital cTBS.

The *upper* panel of Fig. 4 plots the effect of cTBS for occipital and control stimulation on the signal detection theoretic measure of sensitivity d′. We found that occipital cTBS significantly decreased d′ [$t(8) = 2.94$, $P = 0.019$, two-tailed] while control cTBS did not have a significant effect on it [$t(8) = 2.01$, $P = 0.079$, two-tailed]. Critically, a paired-sample *t*-test demonstrated that, compared with control stimulation, occipital cTBS significantly decreased d′ [$t(8) = 2.65$, $P = 0.029$, two-tailed]. These statistics were based on computing the percent change from the pre-cTBS to the post-cTBS session. In a control analysis we performed a repeated-measures ANOVA with factors time of test (pre- or post-cTBS) and condition (occipital or control stimulation). We found a main effect of time of test [$F(1,8) = 8.4$, $P = 0.02$] such that d′ was lower in the post-cTBS sessions. No main effect of condition was present [$F(1,8) = 0.97$, $P = 0.35$]. Critically, there was a significant interaction between time of test and condition [$F(1,8) = 7.82$, $P = 0.023$] mirroring the effect uncovered by the paired-sample *t*-test above and confirming that occipital cTBS led to a larger decrement in d′ than control cTBS.

Next, we investigated subjects' confidence ratings (Fig. 4, *lower* panel). We found that cTBS did not significantly modulate overall confidence after either occipital stimulation [$t(8) =$

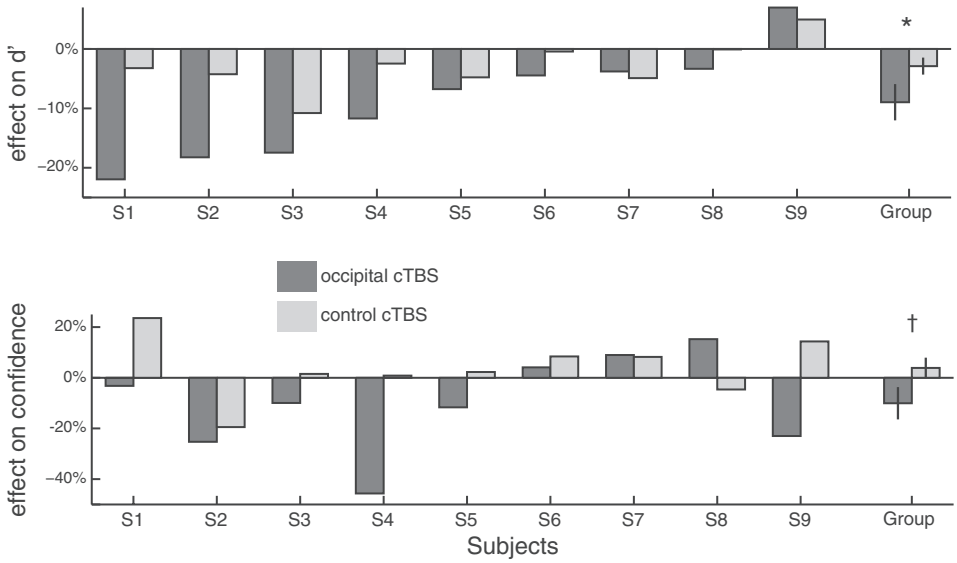

Fig. 4. Effect of cTBS on performance sensitivity d′ and propensity for high-confidence responses. Occipital cTBS led to a significantly higher decrease in d′ compared with control cTBS ($P = 0.029$, two-tailed). There was also a trend for occipital cTBS to lower subjects' propensity to use high confidence, although that effect did not reach significance ($P = 0.073$, two-tailed). Error bars represent SEM. †$P < 0.1$, *$P < 0.05$.

1.58, $P = 0.15$, two-tailed] or control stimulation [$t(8) = -0.97$, $P = 0.36$, two-tailed]. However, a direct comparison between occipital and control cTBS showed a trend for a lower confidence after occipital cTBS [$t(8) = 2.06$, $P = 0.073$, two-tailed], indicating that occipital cTBS may have decreased not just subjects' objective ability to perform the task, but also the certainty in their decisions. As above, the statistics were based on computing the percent change from the pre-cTBS to the post-cTBS session. In a control analysis we performed a repeated-measures ANOVA with factors time of test (pre- or post-cTBS) and condition (occipital or control stimulation). We found no main effect of time of test [$F(1,8) = 0.15$, $P = 0.71$], but a main effect of condition [$F(1,8) = 11.84$, $P = 0.01$] reflecting slightly higher overall confidence in the control sessions (mean %high confidence responses = 58%) than in the occipital session (mean %high confidence responses = 52%). Critically, there was a trend for an interaction between time of test and condition [$F(1,8) = 4.65$, $P = 0.063$] mirroring the effect uncovered by the paired-sample t-test above. We further split the results for d′ and confidence by contrast level (Table 1). No clear relationship emerged relating the effect of cTBS to contrast level.

The above results for d′ and confidence ratings extend our findings from *experiment 1* by confirming that occipital cTBS had an effect on perception. We then turned to our next question regarding whether cTBS had a greater effect on the signal strength or variability of the perceptual signal.

Since occipital cTBS led to lower d′, the effect was likely due to either signal strength decrease or signal variability increase (or both). A priori considerations related to neural excitability and the fact that we observed a trend for a confidence decrease with occipital stimulation pointed toward a likely effect on signal strength (see Fig. 3*B* and explanation above). To formally test this intuition, we performed model fitting by estimating the distance between the peaks of the signal detection distributions (that is, the signal strength, μ), as well as their variability (that is, the signal variability σ; see METHODS). For each subject, we obtained a fitted value for μ and σ for the pre- and post-cTBS sessions for both the occipital and control conditions. A repeated-measures ANOVA on the signal strength μ showed no main effects of time of test [$F(1,7) = 0.23$, $P = 0.65$] or condition [$F(1,7) = 0.05$, $P = 0.84$]. Critically, there was a significant interaction between time of test and condition [$F(1,7) = 10.98$, $P = 0.013$] showing that, compared with control cTBS, occipital cTBS significantly decreased the signal strength (Fig. 5, *upper* panel). A similar repeated-measures ANOVA on the signal variability σ also showed no main effect of time of test [$F(1,7) = 0.17$, $P = 0.7$]. The critical test of the interaction between time of test and condition showed only a trend [$F(1,7) = 4.28$, $P = 0.078$], such that occipital cTBS decreased the variability of the signal more than control cTBS (Fig. 5, *lower* panel). Note that a main effect of condition could not be computed because of the modeling restriction of fixing pre-cTBS variability to 1 (see METHODS). Interestingly, the effect on the variability of the signal was in the opposite direction of what one would expect if occipital cTBS lowered d′ by increasing the variance (see Fig. 3*B*). Therefore, it appears that the main effect of occipital cTBS in this experiment was to decrease the perceptual signal

Table 1. *Effects on d′ and confidence for each contrast*

	d′						Confidence					
	Low contrast		Medium contrast		High contrast		Low contrast		Medium contrast		High contrast	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Occipital cTBS	−7.22	7.27	−18.3	6.3	0.37	5.91	−0.52	2.02	−3.87	2.4	−3.7	2.44
Control cTBS	−3.91	10.45	−7.23	3.61	2.82	5.73	3.21	2.69	2.33	2.26	1.48	1.33

Values are average %change from pre- to post-continuous theta burst stimulation (cTBS) for signal detection theory measure of performance sensitivity (d′) and confidence for each contrast level (low, medium, and high). SE, standard error of the mean.

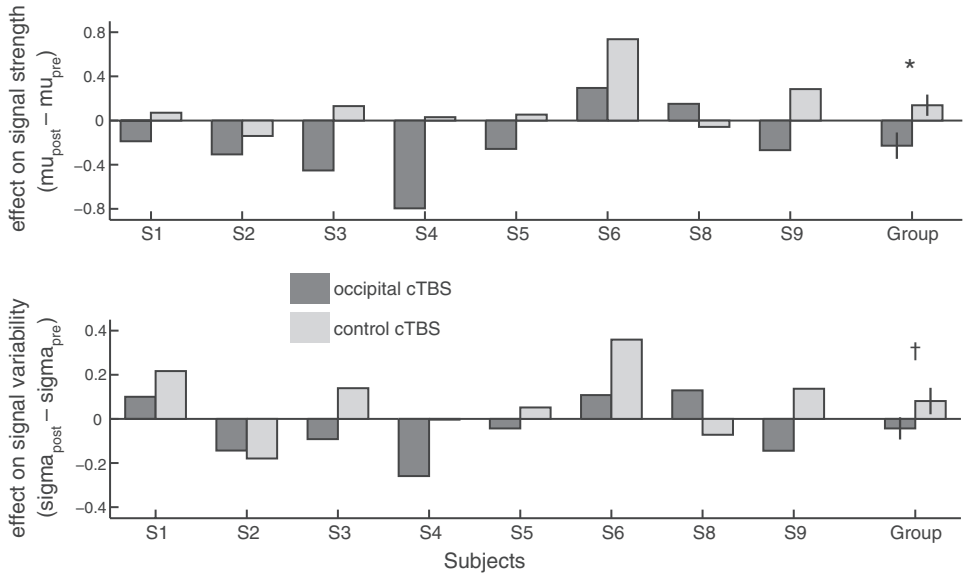

Fig. 5. Effect of cTBS on the perceptual signal (μ) and its variability (σ). We estimated the effect of cTBS on the separation and variability of the internal distributions (see Fig. 3*B*). Since *subject S7* had a very high number of high-confidence responses, the estimated values of μ and σ were highly variable, and the subject was excluded from this analysis. Overall, compared with control stimulation, occipital cTBS decreased the strength of the signal (μ; $P = 0.013$), and not only did it not increase its variability, but there was actually a trend for decreased variability ($P = 0.078$). Thus the behavioral impairments reported in Fig. 4 appeared to be due to a signal loss rather than noise increase. Error bars represent SEM. †$P < 0.1$, *$P < 0.05$.

strength, which may have, in turn, led to a smaller or less reliable decrease in the variability of the signal.

DISCUSSION

We investigated the influence of cTBS applied over the occipital cortex on the mechanisms of sensory processing. In *experiment 1* we found that cTBS decreased the resting state functional connectivity between different regions in the early visual cortex. *Experiment 2* extended these findings by demonstrating a behavioral effect on subjects' performance in a visual discrimination task. These findings are consistent with two possibilities: that cTBS reduced the strength of the perceptual signal (lower resting state connectivity could be coupled with lower gain or signal propagation efficiency), or that cTBS increased the variability of the signal processing the visual cortex (lower resting state connectivity could be coupled with more unstable and thus more variable signal). Our psychophysics analyses demonstrated that cTBS impacted mainly the strength of the perceptual signal, with the impact on its variability showing only a nonsignificant trend. These findings shed new light on the mechanisms of theta burst stimulation on neural processing.

Our results on the influence of TMS on the resting state connectivity between separate brain regions extend previous research on the topic of distant aftereffects of TMS (Bestmann et al. 2008; Chouinard et al. 2003; Fox et al. 2012; Garcia et al. 2011; Hampson and Hoffman 2010; Ruff et al. 2009; Zanto et al. 2011). Previous research has shown that using conditioning pulses over distant areas such as the motion sensitive area MT (Pascual-Leone and Walsh 2001), parietal cortex (Silvanto et al. 2009), or frontal eye fields (Silvanto et al. 2006) can affect the propensity of a subsequent pulse over the occipital cortex to elicit phosphenes. Other research has reported remote effects of TMS outside of the visual cortex (Hampson and Hoffman 2010; Ruff et al. 2009). Here we extend this literature by showing that cTBS can lead to changes in resting state functional connectivity between different areas that persist beyond the time of stimulation. Note that some interhemispheric regions in the visual cortex may not have a monosynaptic connection (Saenz and Fine 2010), but previous research has shown that the effects of TMS can extend to regions without a monosynaptic connection to the targeted region (e.g., Ruff et al. 2006).

It is presently unclear what processes allow cTBS to influence connectivity after stimulation is terminated. It is likely that the persistent aftereffects of cTBS were influenced by a variety of factors, including the pattern of connectivity of each region, the size of the regions, and the susceptibility of neurons to long-term potentiation and LTD. We note that an activation of inhibitory networks is likely to result in a local increase in connectivity and is thus unlikely to explain our results. There was significant variability in the pattern of individual results for the resting state functional connectivity between different pairs of regions. One likely reason for such an effect is the presence of variations in brain anatomy and a resulting variability in the amount of direct magnetic stimulation received by each retinotopic region. Understanding how these differences in brain anatomy affect the amount of induced electrical field to each retinotopically defined region of early visual cortex was beyond the scope of this paper, but there has been a lot of exciting work in this direction (Kammer et al. 2001; Salminen-Vaparanta et al. 2012a, 2012b; Thielscher et al. 2010). Instead, here we focused on the functional consequences of stimulation rather than on the precise amount of stimulation induced in each region. Future studies should investigate how the amount of induced electrical field relates to changes in resting state connectivity.

It is currently unclear how exactly a decrease in resting state functional connectivity (which we observed in *experiment 1*) could be related to signal strength impairments (which we observed in *experiment 2*), especially given their large differences in methods and type of analyses. One possibility is that the signal present at earlier stages of the visual system does not propagate as well to downstream areas and gradually loses part of its strength. Another possibility is that what is most affected by TMS is feedback from downstream areas that guides attention and sets the perceptual template. More research is needed to elucidate the mechanisms by which functional connectivity affects the psychological variables of interest.

Experiment 2 suggested that cTBS may act mainly by decreasing signal strength with only a trend of decreasing signal variability. Only the former of these effects can account for the observed decrease in d′, since such a decrease would require an increase rather than a decrease in signal variability. One intriguing possibility is that signal strength and variability were affected in the same direction because their ratio (known as Fano factor) remains roughly constant. Such an effect has been observed in single neurons (e.g., Dean 1981).

In apparent contradiction with our findings on signal strength and variability, recently our laboratory (Rahnev et al. 2012) and others (Miniussi et al. 2010; Ruzzoli et al. 2010; Schwarzkopf et al. 2011) have provided evidence that online TMS may lead to an increase in perceptual variability rather than to signal loss (although see Harris et al. 2008; Ruzzoli et al. 2011 for studies that reached the opposite conclusion). These results highlight the important possibility that different protocols of TMS are likely to affect neurons in a different fashion. While online TMS induces firing in a subset of the neurons in the stimulated area that interferes with signal processing (Allen et al. 2007), offline cTBS is likely to lead to a lowered excitability of the stimulated area (Allen et al. 2007; Huang et al. 2005). Thus it is perhaps not surprising that online TMS and offline cTBS affect the perceptual system in a different fashion. Finally, it is also possible that the effects of TMS interact with the specific task employed in an experiment, which would also explain the heterogeneity of findings.

Our results are in line with previous research that shows that cTBS to the occipital cortex leads to increased PTs (Franca et al. 2006). However, there is less consistency among previous studies on the aftereffects of offline TMS on visual acuity: while some studies found decreased performance (Antal et al. 2002; Kosslyn et al. 1999), others reported increased visual acuity (Thompson et al. 2008; Waterston and Pack 2010). These studies differed from each other in many aspects, including the frequency, intensity, and location of the stimulation, the size and location of the stimuli in the visual field, and the nature of the task. All of these factors make the comparison between the present study and previous research difficult. More systematic research is needed to map out the influences of these factors on the aftereffects of TMS stimulation.

One caveat of our work is that much more research is needed to be able to fully interpret the consequences of the decreased resting state functional connectivity that we observed in *experiment 1*. As we noted above, lowered resting state connectivity may be coupled to divergent effects when a task is presented, but to date no systematic research has been carried out on this issue. Another caveat is that we had a very small sample size in *experiment 1* ($n = 4$), and therefore these results should be interpreted with caution. As we noted above, any inferences for that experiment are therefore better based on the pattern of individual data rather than the fixed-effects group statistics. Future experiments replicating our findings would ideally involve larger sample sizes. It is also important to note that our signal detection theoretic analysis on signal strength vs. signal variability provided only indirect evidence for the aftereffect of theta burst stimulation. This result should be extended by a more direct investigation of the patterns of neuronal activity following cTBS, either in nonhuman animals or with human neuroimaging studies that measure both neural and behavioral effects of cTBS at the same time.

Finally, it is important to note that we only applied cTBS when subjects were at rest with their eyes closed. Previous research has demonstrated that the level of activation during stimulation is an important factor for the behavioral aftereffect of theta burst stimulation (Silvanto et al. 2007). This research has suggested that cTBS has the highest impact on the least active neural population. Thus it remains an open question what pattern of connectivity decreases or increases cTBS would produce if one subpopulation of neurons is active during theta burst stimulation.

In conclusion, we found that theta burst TMS delivered at rest caused a decrease in resting state functional connectivity between several retinotopically defined regions in the early visual cortex. This resting state connectivity decrease led to a decrease in the perceptual signal and nonsignificant trend for a decrease in the signal variability. Therefore, it appears that the aftereffects of offline cTBS extend beyond the site of stimulation and impact its resting state functional connectivity, which can have important and specific consequences for visual processing.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: D.R., F.P.D.L., and H.L. conception and design of research; D.R., P.K., M.M., L.B., and F.P.D.L. performed experiments; D.R., P.K., F.P.D.L., and H.L. analyzed data; D.R., P.K., F.P.D.L., and H.L. interpreted results of experiments; D.R. and P.K. prepared figures; D.R., P.K., F.P.D.L., and H.L. drafted manuscript; D.R., P.K., M.M., L.B., F.P.D.L., and H.L. edited and revised manuscript; D.R., P.K., M.M., L.B., F.P.D.L., and H.L. approved final version of manuscript.

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