Published in final edited form as:

Brain Stimul. 2018 ; 11(4): 789–796. doi:10.1016/j.brs.2018.02.014.

Single pulse TMS to the DLPFC, compared to a matched sham control, induces a direct, causal increase in caudate, cingulate, and thalamic BOLD signal

Logan T. Dowdle^{a,b}, Truman R. Brown^{c,d}, Mark S. George^{a,b,c,e}, and Colleen A. Hanlon^{a,b,d,e}

^aDepartment of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, South Carolina

^bDepartment of Neurosciences, Medical University of South Carolina, Charleston, South Carolina

^cDepartment of Radiology, Medical University of South Carolina, Charleston, South Carolina

^dCenter for Biomedical Imaging, Medical University of South Carolina, Charleston, South Carolina

^eRalph H Johnson Veterans Administration Medical Center, Charleston, South Carolina

Abstract

Background—In the 20 years since our group established the feasibility of performing interleaved TMS/fMRI, no studies have reported direct comparisons of active prefrontal stimulation with a matched sham. Thus, for all studies there is concern about what is truly the TMS effect on cortical neurons.

Objective—After developing a sham control for use within the MRI scanner, we used fMRI to test the hypothesis of greater regional BOLD responses for active versus control stimulation.

Methods—We delivered 4 runs of interleaved TMS/fMRI with a limited field of view (16 slices, centered at AC-PC) to the left DLPFC (2 active, 2 control; counterbalanced) of 20 healthy individuals (F3; 20 pulses/run, interpulse interval:10–15sec, TR:1sec). In the control condition, 3cm of foam was placed between the TMS coil and the scalp. This ensured magnetic field decay, but preserved the sensory aspects of each pulse (empirically evaluated in a subset of 10 individuals).

Results—BOLD increases in the cingulate, thalamus, insulae, and middle frontal gyri ($p < 0.05$, FWE corrected) were found during both active and control stimulation. However, relative to control, active stimulation caused elevated BOLD signal in the anterior cingulate, caudate and thalamus. No significant difference was found in auditory regions.

Corresponding author: Colleen A Hanlon, Ph.D., Associate Professor, Department of Psychiatry and Neurosciences, Medical University of South Carolina, Charleston, SC 29425, hanlon@musc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors declare no conflicts of interest associated with this manuscript.

Conclusion(s)—This TMS/fMRI study evaluated a control condition that preserved many of the sensory features of TMS while reducing magnetic field entry. These findings support a relationship between single pulses of TMS and activity in anatomically connected regions, but also underscore the importance of using a sham condition in future TMS/fMRI studies.

Keywords

frontostriatal; brain stimulation; connectivity; neuroimaging

Introduction

Transcranial magnetic stimulation (TMS) is a unique, noninvasive tool, which can increase or decrease cortical excitability in specific, targeted circuits. This is supported by previous studies using positron emission tomography (PET), which have demonstrated that TMS to the motor or prefrontal cortex can modulate dopamine binding in monosynaptically connected, subcortical areas [1–3]. PET however, requires the use of a radioligand and has limited temporal and spatial resolution. Another approach to examine the causal effects of TMS on cortical-subcortical circuits is interleaved TMS/fMRI. By applying single TMS pulses between the acquisition of functional volumes, it is possible to measure the brief, transient activation via changes in the BOLD signal in the cortical areas beneath the coil and in subcortical afferents in the basal ganglia [4–8]. As with the PET studies, this has been demonstrated in the motor system [6, 9–13] as well as prefrontal cortex [14–16]. While interleaved TMS/fMRI is a powerful tool, there are still several important methodological considerations and concerns about this technique.

Developing a well-matched control condition has been one challenge for interleaved TMS/fMRI. Researchers have explored a number of methods thus far, including positioning the coil at a 90 or 45 degree angle to the scalp [17], stimulation at lower intensities [18], vertex stimulation as a control site [18] and increasing the distance between the coil and the head [19]. While these techniques are all reasonable approximations, there are a number of limitations to consider. For example, positioning the coil at an angle alters the sensation the subject feels on the scalp, and may still allow a substantial portion of the magnetic field to reach the cortex. [20, 21]. Reducing the intensity of the stimulation, even by as much as 40%, can still lead to changes in cortical excitability [22, 23] and the reduction in the sensation and loudness of TMS can be noted by the participant. Vertex stimulation, which retains the TMS sensation and loudness, is promising, but recent work shows it may result in widespread deactivations across brain networks [24]. Stimulation at any cortical site is likely to have an effect, as no brain region is truly ‘silent’ and not part of the connected whole.

The lack of a psychophysically-matched control condition may account for inconsistencies found in the literature regarding the effects of TMS on cortical and subcortical afferents. For example, in the prefrontal cortex, several studies have demonstrated that BOLD signal is observed near the coil [9, 14, 25]. Other studies, however, have failed to find a difference in BOLD signal in the cortical area under the TMS coil, despite observing modulation in cortical and subcortical afferents [7, 15, 26]. Furthermore, while a few previous studies have demonstrated an intensity-dependent effect of TMS on the BOLD signal [6, 25], these

studies did not control for many of the effects of stimulation, and only a limited range of intensities were explored. The development and evaluation of a control condition that incorporates as many of the sensory aspects, but effectively prevents the entry of the magnetic field may resolve some of these disparate findings. Interest in prefrontal areas, specifically the left dorsolateral prefrontal cortex (LDLPFC), stems from its importance as a clinical target. At present, the LDLPFC is the FDA approved treatment site for depression [27] and is being explored as a treatment for other psychiatric conditions, including addiction [28–31] and pain [32–34].

To address these concerns, we evaluated a control condition in which we increased the coil to cortex distance using 3 cm of firm padding. This preserved many of the sensory aspects of the procedure, while considerably reducing the magnetic field. Additionally, we varied the TMS machine output to evaluate potential intensity-dependent effects of TMS on the evoked BOLD signal. This experimental design was used to test the hypothesis that compared to a control, active TMS would selectively elevate the BOLD signal in the LDLPFC (the site of highest clinical relevance) and subcortical targets.

Materials and Methods

Participants and Procedure

Using word of mouth and digital advertising we recruited twenty healthy individuals from the local community (Table 1). Following written informed consent (approved by the Medical University of South Carolina Institutional Review Board), we invited all eligible participants to the Center for Biomedical Imaging for the experimental visit. Exclusion criteria included a history of seizures, head trauma or a loss of consciousness greater than 15 minutes, history of brain surgery or lesions, use of medications that lower seizure threshold, failure to meet typical MRI safety guidelines, current unstable medical illness or past 6-month illicit drug use.

Upon arrival, we identified the target for the TMS stimulation (left dorsolateral prefrontal cortex (DLPPFC), Beam F3 method [35]) and marked on a Lycra® swim cap (Water Gear Inc., Pismo Beach CA, 0.5mm thickness) which remained in place for the duration of the visit. Resting motor threshold (rMT) was then determined using the same Magstim figure-of-eight coil and a Magstim SuperRapid capacitor (Magstim Inc.) that were subsequently used for the interleaved TMS procedure. rMT was found by modulating the stimulator output until a value resulted in a thumb twitch in 5 out of 10 trials [36]. The average rMT was 68.3% of the machine output ($\pm 8.6\%$).

Interleaved TMS Delivery

Participants were then positioned supine on the bed of the MRI scanner with their head placed securely in a 12 channel head coil (RAPID Biomedical [Rimpar, Germany]) with a built in TMS coil mount [37]. We then placed compressible padding on either side of the head to reduce motion during MRI acquisition. For the “active” stimulation condition, we placed the TMS coil over the marked location on the swim cap (Figure 1A, B). For the “control” stimulation condition, we placed 3cm of firmly compressed open-cell reticulated

foam padding between the TMS coil and the head (Figure 1C). Increasing the distance, d , dramatically weakens the strength of the magnetic field, B , as approximated by the function, $B(d) = 1.05e^{-0.036d}$ ([38], adapted from [39]). With this equation, a 3cm increase in coil to cortex distance results in a 66% reduction in the strength of the magnetic field. This value is in agreement with prior work showing that each millimeter of added distance from the scalp is equivalent to a 3% reduction in stimulator output [40, 41], though this linear approximation is derived from more typical distances (i.e. 10 mm) [42]. Each participant received 4 interleaved TMS/fMRI runs (two active and two control, 20 TMS pulses per run, presented in a counterbalanced order, Figure 1D). The order of the interpulse interval was randomized prior to study initiation to be 10, 13 or 15 seconds. Onsets were set as a list in E-Prime software (Psychology Software Tools, Pittsburgh, PA), which counted each TR, using TTL pulses produced by the scanner. When the appropriate TR was reached, a TTL pulse was sent to the MagStim TMS device, triggering each TMS pulse during a gap (ie. after 900ms) between volumes (see MRI acquisition). Correct stimulation timing was confirmed by the absence of TMS firing artifacts during volume acquisition. When switching from active to control stimulation, we told participants that we were validating the position of the TMS coil. For all runs, the lead author varied the stimulator output (randomized prior to study initiation) by hand to values between 90 to 120% (10% steps) of each participant's rMT. Each change was made during the 10–15 second gap between TMS pulses. Correct timing was ensured through the use of a checklist and stopwatch. For the primary study, we evaluated the integrity of the control condition by asking each participant to state whether a given run felt more or less painful than the proceeding run. Of the individuals stating that they perceived a difference, 2 individuals reported that the control runs were slightly more painful and 3 reported that the active runs were more painful.

Sub-study evaluating the sensory aspects of active and control stimulation

To better quantify the sensory aspects of the stimulation, ten participants were invited back in for a repeat visit (4 male, Age: 26.1 ± 3.5 , rMT: 69.9 ± 8.8). Four sessions of TMS were performed, 2 active and 2 control, with the order fully counterbalanced. Each session of TMS contained 10 pulses, with the amplitude varied throughout the acquisition, as in the primary experiment above. After each session, the participants were asked a series of questions on a scale of 0 to 10: "Overall, how painful was the last session?", "Overall how unpleasant was the last session?", "Overall, how intense and strong were the TMS pulses in the last session?" and "Overall, how loud were the TMS pulses during the last session?". For the 2nd and subsequent TMS runs, they were also asked "Did this TMS session feel the same or different from the previous session?" and asked to rate their confidence on that decision, again on a scale of 0 to 10. After the end of the last session, each participant was told that the purpose of this study was to determine if they received an active stimulation or a stimulation condition wherein the coil was positioned away from the head using padding (i.e. control stimulation). They were then asked to guess, for each session, whether they thought they received active or control stimulation. Each sensory aspect (pain, unpleasantness, intensity and loudness) was analyzed in SPSS using mixed modeling with condition (real vs sham), time, and the condition*time interaction as predictors. Individual subject intercepts and time slopes were entered as random effects in the model. The model employed restricted maximum likelihood estimation (REML) and the covariance structure

was specified as “unstructured”. The collective accuracy of each participants guess was entered into a contingency analysis using Fisher’s exact test to determine performance compared to chance. Confidence for correct guesses and incorrect guesses was averaged.

MRI acquisition

A Siemens 3T TIM trio scanner (Siemens, Erlangen, Germany) and 12 channel head coil was used for all imaging. For both active and control conditions anatomical images (T1 weighted, MPRAGE, 1mm isotropic, 192 slices per slab, TR 1620 ms TE 2.26 ms), a field map (3.4×3.4×3.0 mm, 43 slices, TE₁ 4.6 ms, TE₂ 7.06 ms) and a whole brain T2* weighted anatomical image (3.4×3.4×1.8 mm, 63 slices, TR 3470ms, TE 23 ms) were acquired before the interleaved TMS/fMRI procedure (3.4×3.4×4.0mm, 16 slices, TE 23 ms, TR 1000ms, Flip Angle 60 degrees). The 23 ms echo time was used to reduce susceptibility artifacts, as well as to improve comparability to prior work [14, 15, 43, 44], which used similar values. The short TR in the interleaved TMS/fMRI acquisition was used to better capture the TMS response, but required reducing the number of slices. These limited field of view (FOV) data were acquired with a negative pitch from the AC-PC line (Figure 1A, 2). All 1000ms TR consisted of 900ms of volume acquisition, followed by a 100ms gap, during which the MRI was inactive. Each TMS pulse is triggered to occur at the beginning of this gap, identical to previous work from our group [10, 14].

Imaging data preprocessing

SPM12, running in MatLab 2012a (The MathWorks Inc.), was used for data preprocessing. For each participant, the limited FOV T2* images were coregistered to the whole brain T2* image using the mutual information algorithm in *Coreg: Estimate*. Next, field map derived voxel displacement maps were calculated, and coregistered to the whole brain T2* image (*VDM Toolbox*). *Realign and Unwarp: Estimate and Reslice* was then used to align volumes across time and reduce image distortion. The high resolution MPRAGE was processed through unified segmentation (*Segment*), which simultaneously derives tissue masks (used to mask out the skull with *ImCalc*) and the nonlinear deformations required to warp images into standard space. The full brain T2* images from both active and control sessions were realigned to skull stripped anatomical images, and the limited FOV images were kept in register (*Coregister: Estimate*) and nonlinear deformations were applied (*Normalise: Write*). At this point any transient, single slice artifacts were removed from the normalized data using the default settings of 3dDespike from AFNI (average percent of big edits: 0.44±0.19, for temporal signal to noise ratio image see Supplemental Figure 1). Data were smoothed using an 8mm FWHM Gaussian kernel (*Smooth*). Estimated motion parameters were examined and no subject exceeded the movement threshold of one voxel.

Statistical analyses

The preprocessed data was used for within-subject, general linear modeling. Each level of TMS machine output was modeled separately as a series of instantaneous events, convolved with the canonical double gamma hemodynamic response model provided with SPM12. For nuisance regressors, we used a set of expanded motion parameters. These included the 6 rigid body parameters from SPM’s Realign and Unwarp, their derivatives and the square of the original and derivatives [45]. To remove low frequency drift, we used a high pass filter of

45 seconds and applied SPM12's FAST model to account for autocorrelations. To determine if a linear intensity dependent effect could be found, a separate subject-level model was used. This model was identical to the above, except all TMS onsets were entered as a single vector, with 1st order parametric modulation corresponding to the intensity entered as a second column in the design matrix. This modulates the height of each TMS event by the intensity, and was used to determine if a linear effect is present. **Whole volume analysis.** For group level analysis, a factorial design was used (active vs control X Machine Output) which included eight contrast maps per subject (4 levels of machine output for both active and control). For intensity dependent effects, the subject-specific contrasts corresponding to the positive linear effect during active and control stimulation were entered into a two sample t-test. **Regions of Interest.** A region of interest (ROI) analysis was also performed using anatomically defined ROIs motivated by previous literature. The preprocessed data was converted to units of percent signal change using the CONN toolbox, version 16.b ([46], for the toolbox: www.nitrc.org/projects/conn). Processing was limited to identical high pass filtering and movement parameter regression. The following ROIs were generated from the WFU Pick Atlas [47–49] and Oxford Thalamic connectivity atlas [50, 51]: caudate, putamen, left middle frontal gyrus (LMFG, area under the coil) RMFG, anterior cingulate cortex (ACC), superior temporal cortex (auditory regions, positive control region), globus pallidus and prefrontal zone of the thalamus (Figure 3). Except for the left and right MFG, we chose bilateral ROIs to best capture ipsi- and contralateral projections. Time course extraction was completed using MarsBar [52]. Time courses were exported to Excel (Office 365, Microsoft) and peak activation was extracted by finding the maximum value between 3 and 8 seconds following each TMS pulse. These peaks were averaged (by machine output), yielding a single value for each level of output, ROI and subject. These values were then used in all subsequent statistical analyses, which were performed in GraphPad Prism 7.02 (La Jolla, CA, USA).

Results

Effect of active versus control TMS on whole brain BOLD signal

(Figure 2) Single pulse TMS to the left DLPFC led to elevated BOLD signal in multiple brain regions including the left and right middle frontal gyrus, the bilateral insula, thalamus, superior temporal cortices (auditory cortex), and anterior cingulate (Table 2, Figure 2A, voxelwise $p < 0.05$, FWE corrected). Control TMS to the left DLPFC, however, also led to elevated BOLD signal in many of these regions (Figure 2B, voxelwise $p < 0.05$, FWE corrected).

Relative to the control condition, active TMS led to significantly greater BOLD signal in the caudate & thalamus (Cluster 1, $p < 0.05$, FWE corrected) as well as the anterior cingulate cortices (Cluster 2: $p = 0.025$, uncorrected) (Figure 2, Panel C, cluster forming threshold $p < 0.01$). There were no areas in which control TMS resulted in greater activation (Table 2).

Whole brain intensity dependent effects

There were no areas that showed a significant linear relationship between machine output and the height of the hemodynamic response in either active or control stimulation.

Effects of active versus control TMS in predefined regions of interest

(Figure 3) Active TMS led to a significantly greater BOLD signal in the caudate ($F_{1, 19} = 6.036$, $p=0.0238$, active PSC: 0.616 ± 0.057 , control PSC: 0.544 ± 0.047), the anterior cingulate cortex ($F_{1, 19} = 4.727$, $p=0.0425$, active PSC: 0.359 ± 0.019 , control PSC: 0.313 ± 0.019). There was no significant difference between active versus control TMS in the other regions of interest investigated (left middle frontal gyrus, right middle frontal gyrus, putamen, pallidum, thalamus). There was also no significant difference between BOLD signal in the left auditory cortex, as defined by a region of interest in the superior temporal cortex (positive control region). There were no regions in which control TMS led to greater BOLD signal compared to active stimulation.

Intensity dependent effects in predefined regions of interest

Peak responses from connected ROIs (RMFG, ACC, caudate, superior temporal cortex, putamen, pallidum, thalamus) during stimulation were entered into a linear regression analysis in GraphPad Prism to determine if there was a relationship between machine output and peak response. There was no significant linear relationship between stimulator output in either active or control stimulation.

Sensory Aspects from Sub-study

There was no significant difference between active and sham in pain ($p=0.220$), unpleasantness ($p=0.624$), intensity ($p=0.347$) or loudness ($p=0.451$) (See Supplemental Figure 2). Overall accuracy of guesses was 67.5%. Guessing performance was not significantly better than chance according to Fisher's exact test ($p=0.1086$). The average confidence was 7.33 when participants correctly identified that a session was different, and 7.44 for incorrect identifications.

Discussion

This study presents a direct examination of BOLD signal changes from interleaved TMS/fMRI to the DLPFC compared to a matched control condition that dramatically reduces the entry of the magnetic field. Consistent with previous studies these data demonstrate that DLPFC TMS leads to elevated BOLD signal in the cortex in the vicinity of the coil as well as in cortical and subcortical afferents, including the striatum and thalamus. As an extension of those studies however, this study demonstrates that the control stimulation condition also produces large BOLD signal in many of the same regions. Active prefrontal F3 TMS evoked significantly more BOLD signal than did control TMS in three specific regions, the caudate, the cingulate, and the thalamus – all of which would be predicted by the basic neuroanatomy. The large BOLD response evoked by the control condition in this experiment underscores the need for routine use of control conditions in interleaved TMS/fMRI literature. These data also suggest that our interpretation of data from previous and future studies using interleaved TMS/fMRI should be tempered by the possibility that many of the TMS-evoked changes in BOLD signal may be indirectly related to sensory and attentional aspects of the TMS pulse. This is of particular importance in drawing conclusions about areas that are not expected on the basis of anatomy to be activated in a direct manner by a given TMS target.

As stated above, the data from this study largely replicate observed patterns that have been demonstrated previously, but, importantly the present study reveals that this pattern is largely preserved when the coil is moved 3cm away from the scalp. At this distance, the magnetic field strength has markedly decayed, suggesting that much of widespread activation in traditional interleaved TMS/MRI protocols is due to experimental factors that are indirectly (rather than directly) related to the magnetic-field. The observed similarity between the active and the control condition is likely due to several factors. Within auditory processing regions similar activation is likely due to the startling nature of each pulse, as it is accompanied by a loud pronounced click. The volume of each click is magnified due to the acoustics of the MRI bore and the force on the TMS coil increasing due to the static magnetic field. These increased forces also cause the physical sensation of single TMS pulses (the physical feeling of a 'tap') to be greater than when compared to TMS delivered outside of the MRI environment. Additionally, we ask that participants remain still in response to this startling stimulus, which requires motor control. Together these factors contribute to widespread brain activation that is time-locked to the TMS pulse, but not necessarily related to the circuit that is targeted. Capitalizing on distance, as was done in previous work targeting the intraparietal sulcus [19], appears to be a reliable way to control for the non-specific effects of TMS stimulation. The addition of 3cm of open-cell reticulated foam in the present study controls for the volume of the click, the pressure on the scalp, the angle and position of the physical sensation on the head, yet adds enough distance that the strength of the magnetic field is insufficient to depolarize cortical neurons. Our findings further support the literature highlighting the importance of controlling for the non-specific effects of TMS, which are invariably time-locked to each pulse.

In the present work, the consistent difference observed between active and control TMS in the caudate (a striatal region monosynaptically connected to the DLPFC) buttresses the long-standing statements that TMS applied to the cortex can induce a change in activity in striatal targets. This TMS evoked change in the caudate has been demonstrated previously using multiple modalities including BOLD signal [14] and dopamine binding [3]. The caudate and DLPFC have high functional connectivity [53] and correlated activity as examined by large scale meta-analyses [54]. Another region which was significantly more active during active versus control TMS to the DLPFC was the ACC. As the ACC projects to neighboring regions of the caudate when compared to the DLPFC [55], its increased activity during active stimulation could reflect additional regulatory processes. The final region that showed a difference between active and control stimulation is the prefrontal zone of the thalamus. This region represents the targets of striatal projections prior to looping back to cortical locations [56]. Notably, this effect in the thalamus was observed in the whole brain, voxelwise analysis but not the region of interest analysis, suggesting that the thalamic effects are not as robust as the effects in other ROIs. Finally, it is important to note that no difference ($p=0.43$, percent signal change: active 0.31 ± 0.02 , control 0.30 ± 0.02) was found in the ROI that was used as a positive control region, the superior temporal ROI.

The present study failed to find intensity-dependent effects on BOLD signal in any region examined in active or control stimulation. When linear regression was performed in an exploratory fashion, on a dataset that combined active and control stimulation, only the superior temporal cortex ROI showed a significant linear relationship with stimulator output

($p = 0.0442$). This likely reflects the relatively subtle increase in loudness related to increases in stimulation output, which has been previously been reported [57]. In general, the intensity dependent effects of TMS pulses on evoked BOLD signal have been inconsistent in previous literature. The present findings differ from previous work which found that TMS at higher intensities leads to greater activity when delivered over the motor cortex [6] and in the left DLPFC [25]. These studies differ in several ways from the present study, in that the stimulation profiles, analysis methods and levels of stimulator output differed. In the present work, TMS was delivered at $< 0.1\text{Hz}$, compared to the long (18 or 21 second) 1Hz blocks used in the earlier studies. The early studies also compared these blocks to similar length periods of rest, and used machine output as low as 80%. Together these factors make direct comparisons difficult, though future studies will likely need to use even greater ranges of stimulator output to capture a doseresponse curve.

In order to execute this study with high temporal resolution functional MRI in a time period which did not overburden the participants, we had to make several compromises to the design which limit its generalizability. One limitation in interpreting these results is that the acquired data were restricted to 16 slices that centered around the AC-PC line, as these slices contain the majority of the cortical and subcortical afferents from the DLPFC. The acquisition protocol did not capture the cerebellum or complete volumes of the dorsal aspects of the cortex (including dorsal parietal, primary sensory, motor, and premotor cortices). These areas should be explored in future work, as functional connectivity may underlie the clinical effects of TMS [58] and can involve large scale, whole-brain networks [59]. Additionally, the absence of dose-effects may reflect the range of intensities that were chosen, though previous studies through have also failed to find intensity dependent effects of TMS on evoked BOLD signal under the coil using intensities as high as 150% rMT [26]. Alternatively, detecting these dose-effects may requires more than 40 TMS pulses divided into the 4 different intensities used in the present study. The total number of pulses used in this study is below the number in early work, which used more rapid stimulation profiles, from 0.83 to 10Hz [5, 6, 8, 10, 12, 26, 60]. A future study that delivers more pulses, with a wider range of machine output may be needed to further determine how TMS output leads to changes in the BOLD response. Finally, in retrospect it would have been very valuable to ask the original sample of 20 participants to provide a more comprehensive, quantitative evaluation of the active versus control condition. The follow-up study on a repeated sample of 10 of these individuals provides evidence that the control condition was well matched in the sensory domain, however, future work should improve on this design and evaluate further aspects, such as attention or anticipation.

Conclusions

This study sought to determine if a difference could be found between an active stimulation condition and a control condition which included an additional 3cm displacement of the TMS coil from the participant's scalp – effectively eliminating direct magnetic field effects on cortical excitability. The data reveal strong similarities in evoked BOLD response by active TMS and control TMS. There were however significant differences in the TMS-evoked BOLD signal in the caudate, thalamus, and the cingulate cortex –areas which are strongly predicted by previous research in this area as well as their neuroanatomical

connectivity to frontal-striatal-thalamic loops. The similarity in activation patterns seen under conventional analyses highlights the critical importance of controlling for the non TMS-specific effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institutes of Health (T32 DA007288, R01 DA036617, R21 DA041610, P20 GM109040, UL1 TR001450, F31 DA043330 and P2 CHD086844). Financial support was exclusively provided by NIH, and the NIH had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. The authors also thank James Purl for his invaluable assistance and expertise as an MRI technician, Jayce Doose for his help with technical and engineering concerns. Additionally, the authors would like to thank Oliver Mithoefer and Sarah Hamilton for their help with executing this project and Dr. Jeffrey Borckardt for his statistical advice and expertise.

References

1. Strafella AP, Paus T, Fraraccio M, Dagher A. Striatal dopamine release induced by repetitive transcranial magnetic stimulation of the human motor cortex. *Brain*. 2003; 126(Pt 12):2609–15. [PubMed: 12937078]
2. Cho SS, Strafella AP. rTMS of the left dorsolateral prefrontal cortex modulates dopamine release in the ipsilateral anterior cingulate cortex and orbitofrontal cortex. *PLoS One*. 2009; 4(8):e6725. [PubMed: 19696930]
3. Strafella AP, Paus T, Barrett J, Dagher A. Repetitive transcranial magnetic stimulation of the human prefrontal cortex induces dopamine release in the caudate nucleus. *J Neurosci*. 2001; 21(15):RC157. [PubMed: 11459878]
4. Bohning DE, Shastri A, Lomarev MP, Lorberbaum JP, Nahas Z, George MS. BOLD-fMRI response vs. transcranial magnetic stimulation (TMS) pulse-train length: testing for linearity. *J Magn Reson Imaging*. 2003; 17(3):279–90. [PubMed: 12594717]
5. Bohning DE, Shastri A, McGavin L, McConnell KA, Nahas Z, Lorberbaum JP, et al. Motor Cortex Brain Activity Induced by 1-Hz Transcranial Magnetic Stimulation Is Similar in Location and Level to That for Volitional Movement. *Investigative radiology*. 2000; 35(11):676–83. [PubMed: 11110304]
6. Bohning DE, Shastri A, McConnell KA, Nahas Z, Lorberbaum JP, Roberts DR, et al. A combined TMS/fMRI study of intensity-dependent TMS over motor cortex. *Biol Psychiatry*. 1999; 45(4):385–94. [PubMed: 10071706]
7. Baudewig J, Siebner HR, Bestmann S, Tergau F, Tings T, Paulus W, et al. Functional MRI of cortical activations induced by transcranial magnetic stimulation (TMS). *Neuroreport*. 2001; 12(16):3543–8. [PubMed: 11733708]
8. Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J. Subthreshold high-frequency TMS of human primary motor cortex modulates interconnected frontal motor areas as detected by interleaved fMRI-TMS. *Neuroimage*. 2003; 20(3):1685–96. [PubMed: 14642478]
9. Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J. BOLD MRI responses to repetitive TMS over human dorsal premotor cortex. *Neuroimage*. 2005; 28(1):22–9. [PubMed: 16002305]
10. Bohning DE, Shastri A, Nahas Z, Lorberbaum JP, Andersen SW, Dannels WR, et al. Echoplanar BOLD fMRI of brain activation induced by concurrent transcranial magnetic stimulation. *Investigative radiology*. 1998; 33(6):336–40. [PubMed: 9647445]
11. Bohning DE, Shastri A, Wassermann EM, Ziemann U, Lorberbaum JP, Nahas Z, et al. BOLD-fMRI response to single-pulse transcranial magnetic stimulation (TMS). *J Magn Reson Imaging*. 2000; 11(6):569–74. [PubMed: 10862054]

12. Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J. Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. *Eur J Neurosci.* 2004; 19(7):1950–62. [PubMed: 15078569]
13. Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. *Lancet.* 1985; 1(8437):1106–7. [PubMed: 2860322]
14. Hanlon CA, Canterbury M, Taylor JJ, DeVries W, Li X, Brown TR, et al. Probing the frontostriatal loops involved in executive and limbic processing via interleaved TMS and functional MRI at two prefrontal locations: a pilot study. *PLoS One.* 2013; 8(7):e67917. [PubMed: 23874466]
15. Hanlon CA, Dowdle LT, Moss H, Canterbury M, George MS. Mobilization of Medial and Lateral Frontal-Striatal Circuits in Cocaine Users and Controls: An Interleaved TMS/BOLD Functional Connectivity Study. *Neuropsychopharmacology.* 2016; 41(13):3032–41. [PubMed: 27374278]
16. Hanlon CA, Dowdle LT, Austelle CW, DeVries W, Mithoefer O, Badran BW, et al. What goes up, can come down: Novel brain stimulation paradigms may attenuate craving and craving-related neural circuitry in substance dependent individuals. *Brain Res.* 2015; 1628(Pt A):199–209. [PubMed: 25770818]
17. Osaka N, Otsuka Y, Hirose N, Ikeda T, Mima T, Fukuyama H, et al. Transcranial magnetic stimulation (TMS) applied to left dorsolateral prefrontal cortex disrupts verbal working memory performance in humans. *Neurosci Lett.* 2007; 418(3):232–5. [PubMed: 17467169]
18. Leitao J, Thielscher A, Werner S, Pohmann R, Noppeney U. Effects of parietal TMS on visual and auditory processing at the primary cortical level -- a concurrent TMS-fMRI study. *Cereb Cortex.* 2013; 23(4):873–84. [PubMed: 22490546]
19. Leitao J, Thielscher A, Tunnnerhoff J, Noppeney U. Concurrent TMS-fMRI Reveals Interactions between Dorsal and Ventral Attentional Systems. *J Neurosci.* 2015; 35(32):11445–57. [PubMed: 26269649]
20. Lisanby SH, Gutman D, Luber B, Schroeder C, Sackeim HA. Sham TMS: intracerebral measurement of the induced electrical field and the induction of motor-evoked potentials. *Biol Psychiatry.* 2001; 49(5):460–3. [PubMed: 11274658]
21. Loo CK, Taylor JL, Gandevia SC, McDarmont BN, Mitchell PB, Sachdev PS. Transcranial magnetic stimulation (TMS) in controlled treatment studies: are some “sham” forms active? *Biological Psychiatry.* 2000; 47(4):325–31. [PubMed: 10686267]
22. Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Mazzone P, et al. The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clin Neurophysiol.* 2004; 115(2):255–66. [PubMed: 14744565]
23. Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. *J Physiol.* 1993; 471(1):501–19. [PubMed: 8120818]
24. Jung J, Bungert A, Bowtell R, Jackson SR. Vertex Stimulation as a Control Site for Transcranial Magnetic Stimulation: A Concurrent TMS/fMRI Study. *Brain Stimul.* 2016; 9(1):58–64. [PubMed: 26508284]
25. Nahas Z, Lomarev M, Roberts DR, Shastri A, Lorberbaum JP, Teneback C, et al. Unilateral left prefrontal transcranial magnetic stimulation (TMS) produces intensity-dependent bilateral effects as measured by interleaved BOLD fMRI. *Biological Psychiatry.* 2001; 50(9):712–20. [PubMed: 11704079]
26. Kemna LJ, Gembris D. Repetitive transcranial magnetic stimulation induces different responses in different cortical areas: a functional magnetic resonance study in humans. *Neurosci Lett.* 2003; 336(2):85–8. [PubMed: 12499046]
27. George MS, Lisanby SH, Avery D, McDonald WM, Durkalski V, Pavlicova M, et al. Daily left prefrontal transcranial magnetic stimulation therapy for major depressive disorder: a sham-controlled randomized trial. *Arch Gen Psychiatry.* 2010; 67(5):507–16. [PubMed: 20439832]
28. Barr MS, Farzan F, Wing VC, George TP, Fitzgerald PB, Daskalakis ZJ. Repetitive transcranial magnetic stimulation and drug addiction. *Int Rev Psychiatry.* 2011; 23(5):454–66. [PubMed: 22200135]

29. Bellamoli E, Manganotti P, Schwartz RP, Rimondo C, Gomma M, Serpelloni G. rTMS in the treatment of drug addiction: an update about human studies. *Behav Neurol*. 2014; 2014:815215. [PubMed: 24803733]
30. Gorelick DA, Zangen A, George MS. Transcranial magnetic stimulation in the treatment of substance addiction. *Ann N Y Acad Sci*. 2014; 1327:79–93. [PubMed: 25069523]
31. Grall-Bronnec M, Sauvaget A. The use of repetitive transcranial magnetic stimulation for modulating craving and addictive behaviours: a critical literature review of efficacy, technical and methodological considerations. *Neurosci Biobehav Rev*. 2014; 47:592–613. [PubMed: 25454360]
32. Galhardoni R, Correia GS, Araujo H, Yeng LT, Fernandes DT, Kaziyama HH, et al. Repetitive transcranial magnetic stimulation in chronic pain: a review of the literature. *Arch Phys Med Rehabil*. 2015; 96(4 Suppl):S156–72. [PubMed: 25437106]
33. Lefaucheur JP, Antal A, Ahdab R, Ciampi de Andrade D, Fregni F, Khedr EM, et al. The use of repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) to relieve pain. *Brain Stimul*. 2008; 1(4):337–44. [PubMed: 20633392]
34. Moisset X, de Andrade DC, Bouhassira D. From pulses to pain relief: an update on the mechanisms of rTMS-induced analgesic effects. *Eur J Pain*. 2016; 20(5):689–700. [PubMed: 26471248]
35. Beam W, Borckardt JJ, Reeves ST, George MS. An efficient and accurate new method for locating the F3 position for prefrontal TMS applications. *Brain Stimul*. 2009; 2(1):50–4. [PubMed: 20539835]
36. Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol*. 1994; 91(2):79–92. [PubMed: 7519144]
37. Bohning DE, Denslow S, Bohning PA, Walker JA, George MS. A TMS coil positioning/holding system for MR image-guided TMS interleaved with fMRI. *Clin Neurophysiol*. 2003; 114(11):2210–9. [PubMed: 14580621]
38. George, MS., Bohning, DE., Lorberbaum, JP., Nahas, MDZ., Anderson, B., Borckardt, J., et al. Overview of Transcranial Magnetic Stimulation: History, Mechanisms, Physics, and Safety. In: George, MS., Belmaker, RH., editors. *Transcranial Magnetic Stimulation in Clinical Psychiatry*. 1. Washington D.C: American Psychiatric Publishing; 2007.
39. Bohning DE, Pecheny AP, Epstein CM, Speer AM, Vincent DJ, Dannels W, et al. Mapping transcranial magnetic stimulation (TMS) fields in vivo with MRI. *Neuroreport*. 1997; 8(11):2535–8. [PubMed: 9261822]
40. Stokes MG, Chambers CD, Gould IC, English T, McNaught E, McDonald O, et al. Distance-adjusted motor threshold for transcranial magnetic stimulation. *Clin Neurophysiol*. 2007; 118(7):1617–25. [PubMed: 17524764]
41. Stokes MG, Chambers CD, Gould IC, Henderson TR, Janko NE, Allen NB, et al. Simple metric for scaling motor threshold based on scalp-cortex distance: application to studies using transcranial magnetic stimulation. *J Neurophysiol*. 2005; 94(6):4520–7. [PubMed: 16135552]
42. Stokes MG, Barker AT, Dervinis M, Verbruggen F, Maizey L, Adams RC, et al. Biophysical determinants of transcranial magnetic stimulation: effects of excitability and depth of targeted area. *J Neurophysiol*. 2013; 109(2):437–44. [PubMed: 23114213]
43. Shitara H, Shinozaki T, Takagishi K, Honda M, Hanakawa T. Time course and spatial distribution of fMRI signal changes during single-pulse transcranial magnetic stimulation to the primary motor cortex. *Neuroimage*. 2011; 56(3):1469–79. [PubMed: 21396457]
44. Shitara H, Shinozaki T, Takagishi K, Honda M, Hanakawa T. Movement and afferent representations in human motor areas: a simultaneous neuroimaging and transcranial magnetic/peripheral nerve-stimulation study. *Front Hum Neurosci*. 2013; 7:554. [PubMed: 24062660]
45. Satterthwaite TD, Elliott MA, Gerraty RT, Ruparel K, Loughhead J, Calkins ME, et al. An improved framework for confound regression and filtering for control of motion artifact in the preprocessing of resting-state functional connectivity data. *Neuroimage*. 2013; 64:240–56. [PubMed: 22926292]
46. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect*. 2012; 2(3):125–41. [PubMed: 22642651]

47. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage*. 2003; 19(3):1233–9. [PubMed: 12880848]
48. Maldjian JA, Laurienti PJ, Burdette JH. Precentral gyrus discrepancy in electronic versions of the Talairach atlas. *Neuroimage*. 2004; 21(1):450–5. [PubMed: 14741682]
49. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. 2002; 15(1):273–89. [PubMed: 11771995]
50. Behrens TE, Johansen-Berg H, Woolrich MW, Smith SM, Wheeler-Kingshott CA, Boulby PA, et al. Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging. *Nat Neurosci*. 2003; 6(7):750–7. [PubMed: 12808459]
51. Behrens TE, Woolrich MW, Jenkinson M, Johansen-Berg H, Nunes RG, Clare S, et al. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magn Reson Med*. 2003; 50(5):1077–88. [PubMed: 14587019]
52. Brett, M., Anton, J., Valabregue, R., Poline, J., editors. 8th International Conference on Functional Mapping of the Human Brain. Sendai, Japan: 2002. Region of interest analysis using an SPM toolbox.
53. Choi EY, Yeo BT, Buckner RL. The organization of the human striatum estimated by intrinsic functional connectivity. *J Neurophysiol*. 2012; 108(8):2242–63. [PubMed: 22832566]
54. Pauli WM, O'Reilly RC, Yarkoni T, Wager TD. Regional specialization within the human striatum for diverse psychological functions. *Proc Natl Acad Sci U S A*. 2016; 113(7):1907–12. [PubMed: 26831091]
55. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010; 35(1):4–26. [PubMed: 19812543]
56. Middleton FA, Strick PL. Basal ganglia and cerebellar loops: motor and cognitive circuits. *Brain Res Brain Res Rev*. 2000; 31(2–3):236–50. [PubMed: 10719151]
57. Hanakawa T, Mima T, Matsumoto R, Abe M, Inouchi M, Urayama S, et al. Stimulus-response profile during single-pulse transcranial magnetic stimulation to the primary motor cortex. *Cereb Cortex*. 2009; 19(11):2605–15. [PubMed: 19234068]
58. Fox MD, Buckner RL, White MP, Greicius MD, Pascual-Leone A. Efficacy of transcranial magnetic stimulation targets for depression is related to intrinsic functional connectivity with the subgenual cingulate. *Biol Psychiatry*. 2012; 72(7):595–603. [PubMed: 22658708]
59. Fox MD, Buckner RL, Liu H, Chakravarty MM, Lozano AM, Pascual-Leone A. Resting-state networks link invasive and noninvasive brain stimulation across diverse psychiatric and neurological diseases. *Proc Natl Acad Sci U S A*. 2014; 111(41):E4367–75. [PubMed: 25267639]
60. Blankenburg F, Ruff CC, Bestmann S, Bjoertomt O, Eshel N, Josephs O, et al. Interhemispheric effect of parietal TMS on somatosensory response confirmed directly with concurrent TMS-fMRI. *J Neurosci*. 2008; 28(49):13202–8. [PubMed: 19052211]

Highlights

- BOLD responses in frontostriatal areas distinguish active stimulation from control
- Active and control stimulation lead to similar, widespread patterns of activation
- Well-matched controls are needed when performing interleaved TMS/fMRI

Fig. 1.

Basic Study Set-Up and Design. A: Approximate scalp location of DLPFC, as defined by EEG 10e20 coordinate F3. Brain section within head image shows approximate extent of data collected. B: An example of the coil position for the active stimulation condition. C: An example of the coil position for the sham control condition in which 3 cm of open-cell reticulated foam padding was placed between the coil and F3. D: Task design for interleaved TMS/fMRI. Active and Control Stimulation were counterbalanced. In each case, a pre-randomized interpulse interval was used and the TMS machine output (intensity) was varied throughout the acquisition.

Fig. 2.

Results from Interleaved TMS/fMRI. For coronal and axial slices the left side of the brain is on the left. Fading of dorsal and inferior aspects show areas not available for analysis due to limited field of view. Panel A shows the group statistical parametric map in response to active stimulation, combined across all TMS machine output levels (threshold: voxelwise $p < 0.05$, FWE). Panel B shows the same map for control stimulation (threshold: voxelwise $p < 0.05$ FWE) which is very similar. Panel C shows the positive effect of Active compared to Control stimulation (cluster threshold: $p < 0.01$, uncorrected), in which only frontostriatal afferents are significantly more active in active as compared to control stimulation.

Fig. 3.

Peak Responses in Regions of Interest. The center whole brain shows the ROIs used. All ROIs are bilateral, with the exception of the right middle frontal gyrus (Contralateral to TMS coil) and left middle frontal gyrus (Area Under TMS coil; inset). Both the anterior cingulate cortex and the caudate show a significant main effect of treatment (active vs control stimulation). * = $p < 0.05$.

Table 1

Demographics

N	20 (14 females)
Age	26.8±4.9
Race	17 Caucasian, 3 AA
Education	17.9±3.0 years
Resting Motor Threshold	68.3±8.6

Table 2

Results from GLM analyses for active and control stimulation.

Cluster Statistics		Cluster Locations		Peak Location (MNI)		
PWE-corr	Number of voxels	P _{uncorrected}		x	y	z
<u>Active Stimulation Only</u>						
<0.000	9222	<0.000	B Sup. Temporal, B Mid. Frontal, B Caudate, B Putamen, B Insula	63	-22	17
				-60	-28	17
				9	-16	8
0.001	30	0.012	R Cuneus, R Precuneus, R Angular	33	-52	38
				18	-61	35
				27	-58	35
<0.000	122	<0.000	L Mid. Frontal	-33	47	14
				-24	38	20
				-18	38	5
0.016	4	0.316	R Precuneus, R Mid Cingulate	15	-46	38
0.032	1	0.633	n/a	24	8	26
<u>Control Stimulation Only</u>						
<0.000	7059	<0.000	B Sup. Temporal, B Insula, B Caudate, B Thalamus, B Putamen	-63	-28	20
				63	-25	20
				-63	-37	23
<0.0000	431	<0.000	B Anterior Cingulate, B Mid Cingulate, B Sup. Med. Frontal	6	14	35
				3	23	32
				3	38	14
<0.000	45	0.003	B Mid Cingulate	3	-22	29
				3	-31	26
0.002	17	0.049	R Mid. Frontal	33	47	32
0.025	2	0.484	L Pallidum	-18	-4	-7
0.025	2	0.484	L Mid. Cingulate	-12	-28	41
0.025	2	0.484	R Mid Cingulate	15	-31	38
0.025	2	0.484	R Mid. Frontal	27	44	20

Cluster Statistics			Cluster Locations	Peak Location (MNI)		
P _{FWE-corr}	Number of voxels	P _{uncorrected}		x	y	z
<u>Active greater than Control</u>						
0.254	168	0.025	R Anterior Cingulate, R Sup. Frontal, R Sup. Med., R Mid. Frontal,	15	47	23
0.048	304	0.004	L Thalamus, B Caudate	27	53	26
				6	2	14
				33	11	17
				24	26	26

Active and Sham only stimulation peaks are reported from the voxelwise $p < 0.05$ (FWE corrected) threshold. For the Active greater than Sham comparison, peaks are reported for a threshold of $p < 0.01$, uncorrected, with a cluster size threshold of 100.