Efficacy of Transcranial Magnetic Stimulation Targets for Depression Is Related to Intrinsic Functional Connectivity with the Subgenual Cingulate

Michael D. Fox, Randy L. Buckner, Matthew P. White, Michael D. Greicius, and Alvaro Pascual-Leone

Background: Transcranial magnetic stimulation (TMS) to the left dorsolateral prefrontal cortex (DLPFC) is used clinically for the treatment of depression. However, the antidepressant mechanism remains unknown and its therapeutic efficacy remains limited. Recent data suggest that some left DLPFC targets are more effective than others; however, the reasons for this heterogeneity and how to capitalize on this information remain unclear.

Methods: Intrinsic (resting state) functional magnetic resonance imaging data from 98 normal subjects were used to compute functional connectivity with various left DLPFC TMS targets employed in the literature. Differences in functional connectivity related to differences in previously reported clinical efficacy were identified. This information was translated into a connectivity-based targeting strategy to identify optimized left DLPFC TMS coordinates. Results in normal subjects were tested for reproducibility in an independent cohort of 13 patients with depression.

Results: Differences in functional connectivity were related to previously reported differences in clinical efficacy across a distributed set of cortical and limbic regions. Dorsolateral prefrontal cortex TMS sites with better clinical efficacy were more negatively correlated (anticorrelated) with the subgenual cingulate. Optimum connectivity-based stimulation coordinates were identified in Brodmann area 46. Results were reproducible in patients with depression.

Conclusions: Reported antidepressant efficacy of different left DLPFC TMS sites is related to the anticorrelation of each site with the subgenual cingulate, potentially lending insight into the antidepressant mechanism of TMS and suggesting a role for intrinsically anticorrelated networks in depression. These results can be translated into a connectivity-based targeting strategy for focal brain stimulation that might be used to optimize clinical response.

Key Words: Depression, dorsolateral prefrontal cortex, intrinsic connectivity, MRI, resting state functional connectivity, subgenual, TMS, transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a noninvasive technique that utilizes short, rapidly changing magnetic field pulses to induce electrical currents in underlying cortical tissue (for reviews, see [1–3]). By applying repeated pulses (repetitive transcranial magnetic stimulation) at low frequencies (e.g., 1 Hz), one can suppress underlying cortical activity and high-frequency stimulation (e.g., 20 Hz) can result in excitatory changes (1–3). Further, the effects of TMS can propagate beyond the site of stimulation, impacting a distributed network of brain regions (4–10).

One of the first clinical uses of TMS and its only Food and Drug Administration approved therapeutic indication is high-frequency stimulation to the left dorsolateral prefrontal cortex (DLPFC) for the treatment of medication-resistant depression (11–14). Depression involves a distributed network of cortical and limbic regions, including the DLPFC (especially the left), hippocampus, and subgenual cingulate among others (15,16). Of these, the subgenual region has shown some of the most reproducible abnormalities. The subgenual decreases its activity in response to multiple treatment modalities (Table 1) and is a successful target of deep brain stimulation (DBS) (16–18). Unfortunately, TMS is largely limited to the cortical surface and deeper limbic regions, including the subgenual, cannot be directly or selectively stimulated with traditional stimulation coils. Transcranial magnetic stimulation studies have, therefore, focused on the left DLPFC as one accessible node of this depression network. It has been hypothesized that left DLPFC TMS might have distributed effects on deeper limbic regions such as the subgenual (12,13,19); however, combined TMS imaging studies designed to investigate this hypothesis have produced conflicting results (20–34). It therefore remains unclear how TMS to the DLPFC exerts its antidepressant effect.

Paralleling our limited understanding of the antidepressant mechanism of TMS, its therapeutic efficacy, while statistically significant, also remains limited (11–14). One problem known to contribute to limited average clinical efficacy is difficulty identifying the appropriate stimulation target in the left DLPFC (12,35–38). The Food and Drug Administration approved Neurotronics Neurostar protocol, along with the majority of TMS depression studies, identifies the left DLPFC stimulation site by moving 5 cm anterior to the motor cortex along the curvature of the scalp (11–14,39). However, this technique frequently misses the DLPFC (37,38). Alternative approaches to DLPFC target identification have been examined, in-
including standardized electroencephalogram electrode positions (40), a variety of anatomical magnetic resonance imaging (MRI) coordinates focused around Brodmann areas (BA) 9 and 46 (35,36,41), and individualized hypometabolic foci (42–44) (Table 1). These alternative targeting strategies have not led to substantial clinical improvements beyond the 5 cm approach; however, data from these studies suggest that some DLPFC stimulation sites are more effective than others (12,35,36,42). Unfortunately, it remains unclear why some sites are more effective, making it difficult to capitalize on this information to optimize target selection or clinical effect.

In the current study, we hypothesized that previously reported differences in clinical efficacy of different left DLPFC stimulation sites are related to differences in the connectivity of these sites to deeper limbic regions, especially the subgenual cingulate. We tested this hypothesis using intrinsic (resting state) functional connectivity MRI, a powerful imaging technique that utilizes correlations in spontaneous fluctuations in the blood oxygen level-dependent signal to assess functional relationships between regions (45–47). We first examined a large cohort of normal subjects to detect subtle differences in connectivity between adjacent regions, then confirmed these findings in a smaller cohort of patients with major depressive disorder.

**Methods and Materials**

Full methodological details can be found in Supplement 1. Two datasets collected at different sites were used in the present analyses. The first consisted of 98 healthy right-handed subjects (48 male subjects, ages 22 ± 3.2 years [mean ± SD]). The second dataset consisted of 13 right-handed subjects with major depressive disorder (3 male subjects, mean age 40.2 years, mean Hamilton Depression Rating Scale [HAM-D] 23.8) and 11 healthy control subjects (5 male subjects, mean age 29 years, mean HAM-D .4). These cohorts differed in age, gender ratio, and MRI scanner parameters and therefore cannot be directly compared to look for cohort differences; however, they can be used to test for reproducibility across cohorts. All subjects completed one or more resting state functional connectivity magnetic resonance imaging (fcMRI) scans. Functional connectivity magnetic resonance imaging data were processed in accordance with the strategy of Fox et al. 2005 (48) as implemented in Van Dijk et al. (47), including global signal regression. An a priori region of interest (ROI) was defined in the subgenual cingulate cortex (Figure S1 in Supplement 1) based on coordinates from prior studies showing reductions in subgenual activity tied to antidepressant response (17,23,24,49–52) (Table 1). Additionally, a priori ROIs were defined in the left DLPFC based on coordinates previously used or proposed as TMS targets for depression (Table 1) (25,35–37,41,42,53,54).

Three different analyses were used to relate functional connectivity of various left DLPFC TMS sites to previously reported clinical efficacy: 1) paired comparison of functional connectivity between two TMS sites previously shown to differ in clinical efficacy (35,36); 2) correlation between functional connectivity and clinical efficacy as predicted by a previously reported equation (36): HAM-D drop =
Effects of TMS in the medial prefrontal cortex (subgenual. Sites with strong physiological data showing distribution. Electrode method, showed the weakest anticorrelation with the marks, including the 5 cm method and the electroencephalogram comparisons. Stimulation sites relying on external skull-based landmarks, including the 5 cm method and the electroencephalogram. Functional connectivity between pairs of coordinates from prior studies reporting that one coordinate was clinically superior to another for producing an antidepressant effect. In the first study (Figure 2A), Herbsman et al. (36) recorded the stimulation coordinates from 54 subjects treated with the 5 cm method. They averaged the stimulation sites for responders (−46, 23, 49) and showed this was anterior and lateral to the average stimulation site for nonresponders (−41, 17, 55). Despite the fact that these coordinates were very close to one another, significant differences in functional connectivity were apparent (Figure 2B). The more effective stimulation site was significantly more anticorrelated with the subgenual cingulate compared with the less effective site (Figure 2C, p < .005). In the second study (Figure 2D), Fitzgerald et al. (35) targeted a specific anatomical coordinate (−46, 45, 38) based on evidence from the depression neuroimaging literature and showed (in secondary analyses) that this was superior to the standard 5 cm target (−41, 16, 54 from our analysis). The voxelwise distribution of significant differences in functional connectivity between these two targets (Figure 2E) is similar to that in Figure 2B, although more robust, given the larger separation in the DLPFC coordinates. Also similar to the comparison using the Herbsman et al. (36) coordinates, the more effective stimulation site was significantly more anticorrelated with the subgenual cingulate compared with the less effective site (Figure 2F, p < .0001).

We combined results across these two pairwise comparisons to generate a single map of voxels showing significant differences in functional connectivity between more effective versus less effective DLPFC stimulation sites (Figure S2 in Supplement 1). Peaks in this map were identified (23 positive, 29 negative) and include the subgenual cingulate in addition to several other regions implicated in depression, including the medial prefrontal cortex, orbitofrontal cortex, subgenual cingulate, insula, thalamus, hypothalamus, and hippocampus (Table S1 in Supplement 1).
efficacy of different DLPFC stimulation sites on a continuous basis. First, we computed the average clinical efficacy expected across a group of subjects based on the coordinates of each stimulation site using an equation empirically derived by Herbsman et al. (36). We then plotted the predicted group-level clinical efficacy of all DLPFC stimulation sites considered in the current study (Table 1) versus the resting state correlation of each site with the subgenual cingulate (Figure S3A in Supplement 1). Similar to the paired comparisons, DLPFC sites with higher predicted clinical efficacy showed stronger anticorrelation with the subgenual ($r = -0.842$, $p < .001$ two-tailed). In fact, anticorrelation with the subgenual cingulate accounted for over 70% of the variance in clinical efficacy as predicted by the Herbsman et al. (36) empirically derived equation.

**Correlation Between fCMRI and Clinical Efficacy from Individual Patients**

Moving beyond estimated group-level clinical efficacy using an equation, we next determined whether the above relationship held true for data from individual patients. To test this, we utilized a published table of left DLPFC stimulation coordinates and changes in the Montgomery-Åsberg Depression Rating Scale for 27 individual patients receiving therapeutic TMS for depression (42). For each patient, we plotted their antidepressant response versus the resting state correlation between their specific stimulation site and the subgenual cingulate (Figure S3B in Supplement 1). Note that resting state correlation values in this analysis are average values across our 98 normal subjects, not values from these specific patients, as no resting state functional magnetic resonance imaging data were collected in this prior study. Despite this limitation, left DLPFC sites with higher clinical efficacy in individual patients again showed stronger anticorrelation with the subgenual ($r = -0.355$, $p < .05$, one-tailed). Interestingly, when applied to this independent cohort, there was not a significant relationship between clinical efficacy measured in individual patients and group-level clinical efficacy as predicted by the Herbsman et al. (36) ($r = .122$, $p > .25$, one-tailed; Figure S3C in Supplement 1). This suggests that anticorrelation with the subgenual captures important variance not captured by the Herbsman et al. (36) equation alone.

**Identification of Optimized TMS Targets**

The above results are potentially of interest for understanding the antidepressant mechanism of TMS (see Discussion), but perhaps more importantly, this information can be directly translated into a method to identify connectivity-based coordinates in the left DLPFC that could serve as an optimized TMS target. For example, the above results suggest that anticorrelation with the subgenual is related to antidepressant response. We can therefore use the subgenual ROI as a seed region and identify the peak anticorrelation in the left DLPFC (Figure 3A). Similarly, the above results provide a map of voxels more functionally connected to effective compared with less effective stimulation sites (Figure S2 in Supplement 1). One can use this map as a weighted seed region (minus the left DLPFC to avoid biasing results and inverted to maintain consistency with the subgenual results) to identify an optimized left DLPFC target ($-38$, $44$, $26$; Figure 3B). Note that despite some difference in the coordinates of the peak anticorrelation, these two maps are very similar both across all gray matter voxels (spatial $r = .630$) and specifically within the left DLPFC (spatial $r = .806$). Interestingly, there were several other nodes besides the DLPFC that were anticorrelated with the subgenual, including parietal cortex/intraparietal sulcus, anterior insula, anterior supplementary motor area, and thalamus, which could potentially serve as novel targets of focal brain stimulation for the treatment of depression (Table S1 in Supplement 1) (55,56).

**Replication of Results in Depression**

Since resting state functional connectivity can differ between normal subjects and patients with depression (57), we confirmed our results in an independent cohort of 13 patients with depression using both our subgenual seed region and our efficacy-based seed map. Similar to normal subjects, we found a significant anticorrelation between the subgenual and multiple left DLPFC TMS targets,
including the optimized targets identified above (p < .05; Figure 4A). In paired comparisons, more effective sites showed a trend toward stronger anticorrelation with the subgenual, and the optimized left DLPFC site was significantly more anticorrelated with the subgenual than the standard 5 cm target (p < .05; Figure 4B). As in normal subjects, there was a robust relationship between clinical efficacy as predicted by the Herbsman et al. (36) equation and anticorrelation with the subgenual (r = −.812, p < .005; Figure 4C). Results were even more robust using our distributed efficacy-based seed map rather than the smaller and noisier subgenual ROI (Figure 4D–F). Similar to the subgenual, many DLPFC targets, including our optimized sites, showed a significant negative correlation with the seed map (Figure 4E). In paired comparisons, more effective sites were significantly more anticorrelated than less effective sites, including the Herbsman et al. (36) regions (p < .05), the Fitzgerald et al. (35) regions (p < 10^-4), and our new optimized site compared with the standard 5 cm target (p < 10^-6). Finally, there was a highly significant relationship between predicted clinical efficacy and correlation with our efficacy-based seed map (r = −.875, p < .001).

Analyses were also replicated on the 11 control subjects from the same dataset as the 13 patients with depression (Figure S4 in Supplement 1). There were no significant differences between these control subjects and patients with depression.

Discussion

In the current article, we used a novel connectivity-based approach to gain insight into why some left DLPFC TMS targets have proven more clinically effective than others. We identified robust differences in functional connectivity related to previously reported differences in clinical efficacy, particularly anticorrelation with the subgenual cingulate. We then demonstrated how one could translate this information into a connectivity-based targeting technique to identify coordinates in the left DLPFC that could potentially be stimulated to optimize clinical response.

These results are likely relevant to understanding network models of depression, the antidepressant effect of TMS, and the functional relevance of intrinsic anticorrelations in resting state functional magnetic resonance imaging. Most importantly, the current results suggest that the clinical efficacy of focal brain stimulation might be optimized by targeting based on connectivity, a concept that remains to be tested in clinical trials but could find broad applicability across a number of diseases and stimulation techniques.

Relevance to Network Models of Depression

Depression is becoming increasingly recognized as a network disorder associated with alterations in a distributed set of regions, including DLPFC (especially left), medial prefrontal, orbitofrontal, subgenual cingulate, insula, thalamus, hypothalamus, and hippocampus (15,16). Of these regions, the left DLPFC and the subgenual cingulate have received the most attention due to the consistency of their depression-related abnormalities, their modulation with treatment across a range of therapies, and their use as targets of focal brain stimulation (58). Although depression functional imaging studies have produced heterogeneous results (16,59–61), on average, the abnormalities in these two regions have been opposite one another (58). The subgenual has been observed to be hyperactive in depression and a decrease in this hyperactivity is associated with antidepressant response (16,17,58) (Table 1). Conversely, the left DLPFC tends to be hypoactive in depression and an increase in activity is associated with antidepressant response (58,59). Consistent with this dichotomy, lesions of the ventral medial prefrontal cortex can improve depression, while lesions of the dorsal lateral prefrontal cortex can exacerbate it (62).

The current finding that the subgenual and DLPFC are intrinsically anticorrelated during the resting state mirrors this dichotomy and suggests that there is a link between the depression-related abnormalities in these two regions. There are several implications of this result. First, observed depression-related abnormalities in one region could theoretically be due solely to pathology in the opposing region. Primary hyperactivity in the subgenual might result in secondary hypoactivity of the DLPFC without anything being abnormal in the DLPFC and vice versa. Second, this anticorrelation could mediate compensatory responses. The DLPFC could increase its activity in response to subgenual hyperactivity in an attempt to suppress or normalize activity in this region, a mechanism that could explain the occasional finding of DLPFC hyperactivity in depression (15,59,60). Finally, focal inhibition/excitation of one region could be expected to, respectively, enhance/suppress activity of the other region. Indeed, DBS of the subgenual (which suppresses activity locally) results in an increase in activity in the DLPFC (17).

While the above discussion focused on the subgenual and the DLPFC, it is important to remember that the current results include several other regions previously implicated in the pathology of depression (15,61). Our results suggest two anticorrelated groups of regions. The first consists of the subgenual, medial prefrontal, superior frontal, hippocampus, posterior cingulate/precuneus, middle temporal gyrus, and cerebellar tonsils, while the second consists of the DLPFC, anterior insula, dorsal anterior cingulate/presupplementary motor area, thalamus, DLPFC, and parietal cortex.
**Understanding the Antidepressant Mechanism of TMS**

There has been much research into the antidepressant mechanism of DLPFC TMS in the hopes that this knowledge would facilitate optimization of the effect and improve clinical utility. Many hypotheses have been proposed (12,63); however, one idea that has been pursued aggressively is the propagation of TMS effects through anatomical connections to deeper limbic regions (12). A number of groups have attempted to localize the remote effects of DLPFC TMS by pairing it with neuroimaging techniques both in normal subjects and patients with depression. A full review of these heterogeneous results is beyond the scope of this article (see Fox et al. [65] for discussion), the current results add information to be considered in the ongoing debate. First, the fact that the resting state anticorrelation between the subgenual and DLPFC is recapitulated in patterns of pathological abnormalities seen in depression provides additional evidence that anticorrelations may reflect functionally meaningful relationships. Second, the focal brain stimulation interventions used in depression might serve as a causal test of the functional importance of anticorrelations. If stimulation/inhibition of one node suppresses/augments the activity of the anticorrelated node in a spatially specific manner and in proportion to the strength of the anticorrelation, this would support the biological importance of anticorrelations.

An interesting issue is determining how anticorrelations observed with resting state fcMRI in the setting of a preprocessing step termed global signal regression (47,64–67). This processing can improve the specificity of resting state correlations and the correspondence with anatomy (65); however, there are mathematical concerns that anticorrelations could emerge as processing artifact. While the technical issues surrounding processing strategy and anticorrelations are beyond the scope of this article, the current results add information to be considered in the ongoing debate.

**Relevance to the Debate Surrounding Anticorrelations**

There has been substantial debate surrounding the appropriate interpretation of anticorrelations observed with resting state fcMRI in the case of the subgenual and DLPFC, the anticorrelation is unlikely to be the result of a round processing strategy and anticorrelations are beyond the scope of this article. The current results add information to be considered in the ongoing debate. First, the fact that the resting state anticorrelation between the subgenual and DLPFC is recapitulated in patterns of pathological abnormalities seen in depression provides additional evidence that anticorrelations may reflect functionally meaningful relationships. Second, the focal brain stimulation interventions used in depression might serve as a causal test of the functional importance of anticorrelations. If stimulation/inhibition of one node suppresses/augments the activity of the anticorrelated node in a spatially specific manner and in proportion to the strength of the anticorrelation, this would support the biological importance of anticorrelations.
of direct inhibitory connections. Monkey track-tracing studies sug-
ggest that there are no direct anatomical connections between BA46
and BA25 (68,69). However, there are direct anatomical connec-
tions between the subgenual (BA25) and the anterior insula and
mediodorsal nucleus of the thalamus, both of which are anticorre-
lated with the subgenual in the current analysis. Previous studies
have implicated the fronto-insular cortex as a potential node medi-
ating anticorrelations (70), and other studies have suggested the
thalamus, especially the mediodorsal nucleus, as the site of integra-
tion of otherwise separate cortical-subcortical loops (71).

Targeting Focal Brain Stimulation Based on Connectivity

The idea that targets for focal brain stimulation should be se-
lected, at least partly, based on their connectivity to other regions is
not new; however, implementing this strategy in practice has been
difficult and empiric evidence supporting the utility of this ap-
proach has been limited (for review, see [10]). It has been suggested
that stimulation should be targeted to the portion of the DLPFC
with connectivity to deeper limbic regions (12,19). Unfortunately,
the connectivity between the DLPFC and various limbic regions is
complicated even in monkeys (68,69), and the DLPFC is one of the
areas that has expanded the most throughout evolution (54,72). It
has remained unclear which part of the human DLPFC should be
stimulated and which limbic regions are important, even if the
human connectivity between the DLPFC and limbic regions was
well established.

In the current article, we use intrinsic fcMRI with the subgenual
and our efficacy-based seed map to identify left DLPFC TMS coordi-
nates designed to optimize antidepressant response. These coordi-
nates might serve as the basis for a clinical trial; however, this
connectivity-based targeting approach can be taken further. First,
our results suggest the existence of other connectivity-based TMS
targets for depression besides the DLPFC (Figure 3; Table S1 in
Supplement 1). Of these, the cerebellum and parietal cortex have
previously been suggested as potential TMS targets in depression
based on mood effects in normal subjects (56). A recent trial of
low-frequency parietal stimulation failed to show a significant re-
sponse beyond sham (55); however, the present results suggest
that high-frequency stimulation to the peak parietal node anticorre-
lated with the subgenual may be more effective. Second, the
current study reports average group-level coordinates. Although
average coordinates have previously been used in clinical trials of
TMS for depression (35), an advantage of the current targeting
approach is it might be applied at the single subject level. Given
cross-subject heterogeneity in the location of the DLPFC (54), the
full potential of connectivity-based targeting may be realized with
identification of individualized TMS targets tailored to individual
patients. Finally, the current targeting approach is potentially appli-
cable across other diseases and brain stimulation techniques. Cor-
tical correlates of deep brain stimulation sites based on fcMRI could
serve as important TMS targets in Parkinson’s disease, dystonia,
obsessive-compulsive disorder, or any other disease for which DBS
provides clinical benefit (73). The converse of this approach also
holds promise. Specifically, intrinsic fcMRI could be used to identify
optimized DBS sites in individual patients based on connectivity
with distributed cortical networks known to be impacted by dis-
ease.

Limitations and Future Work

The current work was limited in several respects and these limi-
tations suggest important avenues for future research. First, our
results were generated on normal subjects then confirmed in a
small cohort of patients with depression. While this makes it likely
that our findings will further generalize to a larger cohort of patients
with medication-refractory depression undergoing TMS, our results
remain to be confirmed in this specific population. Second, mea-
sures of clinical efficacy in the current article were based on previ-
ously published data and not obtained de novo. Ideally, one would
measure clinical efficacy and resting state functional connectivity in
the same cohort of patients. However, the fact that our connectivity
results in normal subjects predicted clinical efficacy in an indepen-
dent set of patients suggests that future work measuring both
parameters in the same cohort should only increase the strength of
the relationship. Finally, the current findings suggest that the anti-
depressant effect of TMS might be optimized through connectivity-
based targeting; however, this remains a hypothesis. The clinical
utility of this method remains to be tested in a clinical trial.

MDF was supported by National Institutes of Health Grant
R25NS065743. Work on this study was also supported by grants from
the National Institutes of Health and National Center for Research
Resources: Harvard Clinical and Translational Science Center (UL1
RR025758); the Howard Hughes Medical Institute, and the Dana Foun-
dation.

We thank the Brain Genomics Superstruct Project for contributing
data.

AP-L serves on the scientific advisory boards for Nexstim, Neurionix,
Starlab Neuroscience, Allied Mind, Neosync, and Novavision and is
listed as inventor in issued patents and patent applications on the
real-time integration of transcranial magnetic stimulation with elec-
troencephalography and magnetic resonance imaging. All other au-
thors report no biomedical financial interests or potential conflicts of
interest.

Supplementary material cited in this article is available online.

4. Valero-Cabre A, Payne BR, Rushmore J, Lomber SG, Pascual-Leone A
(2005): Impact of repetitive transcranial magnetic stimulation of the
parietal cortex on metabolic brain activity: A 14C-2DG tracing study in
14C-2-deoxyglucose brain metabolism following patterns of high and
low frequency repetitive transcranial magnetic stimulation in the pos-
6. Siebner HR, Bergmann TO, Bestmann S, Massimini M, Johansen-Berg H,
‘virtual lesions’ to functional-network accounts of cognition. Cortex 45:
1043–1049.
(2011): Human brain connectivity during single and paired pulse trans-
9. Lisanby SH, Belmaker RH (2000): Animal models of the mechanisms of
action of repetitive transcranial magnetic stimulation (RTMS): Compar-
manipulating brain connectivity with resting state functional connec-
tivity magnetic resonance imaging (fcMRI) and transcranial magnetic
stimulation (TMS) [published online ahead of print March 19]. Neuroim-
age.
Z, et al. (2007): Efficacy and safety of transcranial magnetic stimulation in
the acute treatment of major depression: A multisite randomized con-


