Using interleaved transcranial magnetic stimulation/functional magnetic resonance imaging (fMRI) and dynamic causal modelling to understand the discrete circuit specific changes of medications: Lamotrigine and valproic acid changes in motor or prefrontal effective connectivity

Using interleaved transcranial magnetic stimulation/functional magnetic resonance imaging (fMRI) and dynamic causal modeling to understand the discrete circuit specific changes of medications: Lamotrigine and valproic acid changes in motor or prefrontal effective connectivity

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A B S T R A C T

The purpose of this study was to use interleaved transcranial magnetic stimulation/functional magnetic resonance imaging (TMS/fMRI) to investigate the effects of lamotrigine (LTG) and valproic acid (VPA) on effective connectivity within motor and corticolimbic circuits. In this randomized, double-blind, crossover trial, 30 healthy volunteers received either drug or placebo 3.5 h prior to interleaved TMS/fMRI. We utilized dynamic causal modeling (DCM) to assess changes in the endogenous effective connectivity of bidirectional networks in the motor–sensory system and corticolimbic circuit. Results indicate that both LTG and VPA have network-specific effects. When TMS was applied over the motor cortex, both LTG and VPA reduced TMS-specific effective connectivity between primary motor (M1) and pre-motor cortex (PMd), and between M1 and the supplementary area motor (SMA). When TMS was applied over prefrontal cortex, however, LTG alone increased TMS-specific effective connectivity between the left dorsolateral prefrontal cortex (DLPFC) and the anterior cingulate cortex (ACC). In summary, LTG and VPA both inhibited effective connectivity in motor circuits, but LTG alone increased effective connectivity in prefrontal circuits. These results suggest that interleaved TMS/fMRI can assess region- and circuit-specific effects of medications or interventions.

1. Introduction

Lamotrigine (LTG) and valproic acid (VPA) are broad spectrum anticonvulsants that are commonly prescribed for their mood-stabilizing properties (Moeller et al., 2009). Many *in vitro* and *in vivo* studies have shown that these drugs modify neuronal excitability through different molecular mechanisms (Xie et al., 1995; Loscher, 1998). LTG has been shown to inhibit voltage-gated sodium channels (Xie and Hagan, 1998) while VPA is thought to alter intracellular signaling cascades and gamma-aminobutyric acid (GABA) levels (Rogawski and Loscher, 2004). Unfortunately, little is known about how these drugs affect neural circuitry in humans.

Transcranial magnetic stimulation (TMS) has been used to examine how anticonvulsant drugs alter motor circuit excitability in healthy participants and patients with epilepsy (Ziemann et al., 1996, 1998, 2002; Manganotti et al., 1999; Boroojerdi et al., 2001). Although motor evoked potentials (MEPs) provide useful information about medication-induced changes in corticospinal physiology, they cannot reveal where a drug acts or how it alters non-motor circuits implicated in attention, mood, or sensory processing.

Some of the limitations inherent to pharmacological TMS studies are addressed by pharmacological functional magnetic resonance imaging (phMRI) studies, which measure blood oxygen level dependent (BOLD) signal changes in response to neuroactive drugs (Tracey, 2001; Borsook et al., 2006). However, phMRI studies typically require activation paradigms that selectively elicit brain activity with cognitive, sensory, or motor tasks performed inside the scanner. A major problem with these activation paradigms is that behavioral performance varies between individuals based on skill level, motivation, and health. These variables can cause inconsistent performance and thus make it difficult to compare BOLD signal changes between healthy volunteers and patients (Wise and Tracey, 2006).

Interleaved TMS/functional magnetic resonance imaging (fMRI) overcomes the drawbacks of either technique in isolation because it uses cortical stimulation, not behavioral tasks, to evoke brain activity that can be imaged with a high degree of spatial and temporal

resolution (Bohning et al., 1999; Bestmann et al., 2004; Sack et al., 2007; Mevorach et al., 2010). Two of our previous studies with interleaved TMS/fMRI show that broad spectrum anticonvulsant drugs diminish local and distributed activity induced by TMS in healthy volunteers (Li et al., 2004b, 2010). However, there are currently no studies that analyze how these drugs affect functional integration between cortical and subcortical regions.

The specific purpose of this study was to use interleaved TMS/fMRI to test our hypothesis that LTG and VPA modify different brain cortical circuits and corticolimbic circuits in humans. We stimulated motor or prefrontal cortex and simultaneously imaged brain activity in healthy participants who had received an oral dose of LTG, VPA or placebo. In a conventional analysis, we previously demonstrated that LTG and VPA had similar inhibitory effect on motor cortex and a different effect on prefrontal cortex (Li et al., 2010). Dynamic causal modeling (DCM) was introduced as a generic method to estimate effective connectivity from functional magnetic resonance imaging (fMRI) data in a Bayesian fashion (Friston et al., 2003). DCM is distinguished from alternative approaches not just by accommodating the nonlinear and dynamic aspects of neuronal interactions, but by framing the estimation problem in terms of perturbations that accommodate experimentally designed inputs. In DCM the causal variables that compose the conventional design matrix become the inputs and the parameters become measures of effective connectivity (Friston et al., 2003; Stephan, 2006; Stephan et al., 2007b, 2010). As such, DCM can be used to measure how neuronal dynamics respond to TMS (Grefkes et al., 2010) and how to drugs alter TMS-specific effective connectivity.

2. Participants and methods

2.1. Participants

In this study, data previously acquired from thirty healthy men aged 18–35 (Li et al., 2010) were analyzed with DCM. All participants were previously given a detailed explanation of the procedure and signed a written informed consent form approved by the Medical University of South Carolina Institutional Review Board (IRB).

2.2. Procedures

Study design: After the initial screening visit, we performed a randomized, double-blind, crossover trial involving three visits at least 1 week apart. After arriving at the laboratory in the early morning, participants were given a single oral dose of 325 mg of LTG or 1250 mg of VPA or placebo. They then waited quietly. Three and half hours after taking the oral pill, combined TMS and fMRI was performed and serum plasma samples were drawn.

Combined TMS and fMRI: Combined TMS and fMRI acquisitions were performed in a Philips 3.0 Tesla MRI scanner (Intera, Philips Medical System, The Netherlands) with an eight-channel SENSE head coil, using a standard gradient echo, echo planar imaging (EPI) fMRI sequence (flip angle = 90, repetition time (TR) = 2.3 s, echo time (TE) 0.032 s, field of view (FOV) 230 mm, 23 3.5 mm thick slices, 0.5 mm gap, Matrix (64×64). The fMRI time series consisted of 342 images preceded by six dummy images. TMS was applied using a Magstim Super Rapid stimulator which generates biphasic electrical pulses of approximately 250 μs duration. The pulses were delivered through a special non-ferromagnetic TMS coil of figure-of-eight design with an 8-meter cable and a room setup identical to prior TMS/fMRI studies from our group (Li et al., 2004b). TMS pulses and the fMRI sequence were interleaved as described before (Shastri et al., 1999). LabVIEW running on a G4 computer was used to control the TMS intensity, as well as light which was used to trigger voluntary thumb movements. A CA-Connector Accessory Enclosure from National Instruments was connected to the TMS and the G4 computer in order to precisely integrate the TMS pulses (or light) within the scan acquisition. We employed a variable, jittered block design with three tasks -five 1-Hz pulses of TMS at 100% of resting motor threshold (RMT), five 1-Hz pulse of TMS at 120% of RMT, or right thumb voluntary movements triggered by a light at 1 Hz. This block order was repeated 17 times during a 786-s scan. The rest time between conditions varied from 8 to 13 s in order to minimize anticipation and to promote novelty. The order of the tasks (i.e., 100% RMT, 120% RMT, and voluntary thumb movement) was randomized within each block with a web-based randomization generator (www.randomization.com). Subjects were instructed to keep their eyes open and to relax their hands while repetitive TMS pulses were delivered using the two different intensities. For the volitional thumb movement task, a light was set in front of subject's face outside of the MRI head coil, which flashed to signal subjects to move their thumb.

TMS Coil Placement in the MRI scanner: Before subjects were placed into the MRI scanner, their resting motor threshold (RMT) was determined with the Magstim stimulator within the scanner. The site of TMS stimulation was located at the motor "hot spot", defined functionally as the point of maximum evoked motor response in the relaxed right abductor pollicis brevis (APB). The RMT was defined as the lowest stimulus intensity that elicited at least five muscle twitches from 10 consecutive stimuli given over the motor hot spot. This RMT determined on the first visit was used as the standard intensity for TMS throughout the three visits. After RMT was determined, the TMS coil was rigidly mounted in the MR head coil with a specially designed TMS coil-holder, adjustable in six directions (Bohning et al., 2003). Subjects wore swim caps on which their individual hot spot was marked to facilitate coil positioning and earplugs to reduce auditory responses. Head holder position and the RMT stimulus intensity were recorded and used for an individual's subsequent visits.

Repeating the method used in the earlier study, the site for stimulation of the left prefrontal cortex was defined as a location 5-cm rostral and in a parasagittal plane from the motor hot spot (George et al., 1995). The TMS coil was repositioned and subjects then re-entered the scanner for the prefrontal cortex TMS scan. The stimulation and acquisition protocol was then identical to the motor cortex scan described above. The order in which TMS was applied over either motor cortex or prefrontal cortex was also randomized across weeks (Li et al., 2010).

2.3. Conventional fMRI data analysis

Individual fMRI data analysis: See conventional analysis in details in the study by Li et al. (2010). These data served as the base for the DCM below.

2.4. Dynamic causal modeling

2.4.1. Connectivity analysis

Dynamic causal modeling (Friston et al., 2003), as implemented in the SPM5 software package, was employed for connectivity analysis. The aim of DCM is to estimate how experimental context influences the capacity of one neural system to exert control over or interact with another. We restricted DCM to the left hemisphere because of data from previous TMS studies (Lee et al., 2003; Li et al., 2004b) and conventional TMS/fMRI data analysis (Li et al., 2010). We defined regions of interest according to the nearest local maximum in relation to group coordinates. Regions of interest were extracted in a sphere region (radius = 6 mm) from the "effects of interest–120%RMT–TMS minus rest" F-contrast ($p<0.001$, uncorrected), and adjusted for effects of 120%RMT–TMS.

2.4.2. Model definition for motor cortex TMS

The motor cortex TMS network model consisted of four left-hemisphere regions which were reported in previous studies

(Bohning et al., 1999; Tekin and Cummings, 2002; Lee et al., 2003; Li et al., 2004b, 2010; Bestmann et al., 2005; Denslow et al., 2005). The four regions were: primary motor cortex (M1, BA4, −29 −16 57), pre-motor region (PMd, BA6, −43 1 48), supplementary motor cortex (SMA, BA6, −3 1 60), and thalamus (Thal, −12 −20 15).

Using SPM5, BOLD signal time courses were extracted from 6-mm spheres centered on subject-specific maxima located within 9 mm of the placebo group maxima for each area in the network model. Using the above regions of interest, individualized dynamic causal models were assembled with identical connectivity within subjects. For the primary network model, information was assumed to travel outward from M1 to other regions (Ganesh et al., 2008; Kasess et al., 2008). We also assumed there were bidirectional connections between the four regions. A model selection approach can be used to compare competing models. The primary DCM model was compared with alternate network models using placebo group data and DCM model comparison procedures to arrive at the best network model (Penny et al., 2004).

In this study, we used Bayesian model selection (BMS) to decide which DCM was optimal. BMS takes into account the relative fit and relative complexity of competing models (Stephan et al., 2007a). For DCM, two suitable approximations are the Bayesian information criterion (BIC) and Akaike information criterion (AIC) (Penny et al., 2004; Stephan et al., 2007a). The decision between competing models is then based on that approximation which gives the more conservative Bayes factor (BF) as formula: $BF_{ij} = P(y/m_i)/P(y/m_j)$. An established convention is to prefer one model over another if the BF is >3 ("positive evidence"). When determining the optimal model for a group of individuals by BMS, it is likely that the optimal model will vary to some degree across subjects. Because model comparisons from different individuals are statistically independent, a group Bayes factor can be computed by multiplying the individual Bayes factors as formula: $GBF_{ij} = \pi BF^{k}_{ij}$ (Stephan et al., 2007a).

For each subject for each task, we performed comparisons between all models and then computed the group Bayes factors (GBFs) across subjects. We additionally evaluated the number of comparisons for which the BF passed the threshold for positive evidence for either of the compared models. These numbers give a "positive evidence ratio" (PER), which serves as a complementary measure of which model is optimal at the group level (Stephan et al., 2007a).

2.4.3. Model definition for prefrontal cortex TMS

The model definition for prefrontal cortex TMS is based on published data from studies of anatomical connectivity in human (Tekin and Cummings, 2002; Li et al., 2004a,b; Schlosser et al., 2008). The prefrontal cortex TMS network model consisted of three regions: left dorsolateral prefrontal cortex (DLPFC, BA9, −39 28 51), medial prefrontal cortex (mPFC, BA9, −7 35 38), and anterior cingulate cortex (ACC, BA25, −3 26 0). These three regions are included in an important fronto-cingulate circuit which had been reported as a significant activation in our previous study (Li et al., 2004a, 2010).

As reported in previous publications of conventional data, bidirectional connections from DLPFC to mPFC and ACC were estimated. The primary DCM model was compared with alternate network models using DCM model comparison procedures applied to data from TMS applied over prefrontal cortex in the presence of LTG to arrive at the best network model (see description for motor cortex TMS).

After estimating parameters of intrinsic connections and modulatory effects on an individual subject level, with SPSS 12.0, the second-level statistics was performed by analysis of variance (ANOVA). The analysis was used to identify main effects of group, which means that connections across the whole network were significantly different between the two drugs and placebo. The null hypothesis of no difference in connection strength between LTG, VPA and placebo was

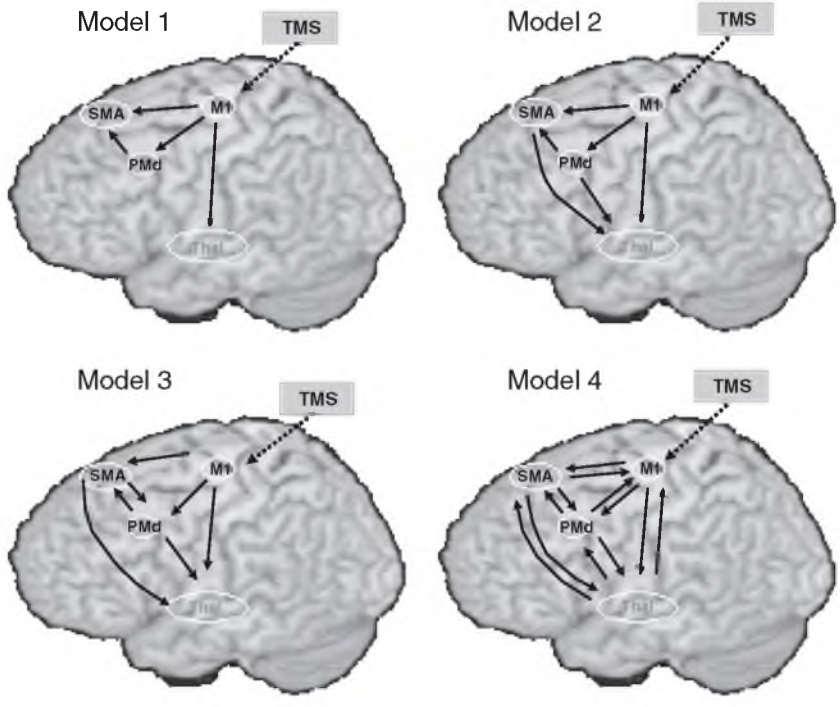

Fig. 1. Model network of interregional connections and experimental inputs for motor cortex experiment. Regions were connected with stimulus inputs entering M1, PMd, SMA and thalamus (black labels indicate cortical region, gray labels indicate non-surface region). The arrows and arrowhead highlight defining organizational features for each network model: Model 1, M1 outputs all other regions and from PMd to SMA; Model 2, M1 outputs all other regions and thalamus inputs from all other region, SMA inputs from PMd; Model 3, M1 outputs to all the others, thalamus inputs from all the others, PMd and SMA bidirectional inputs and outputs. Model 4 is a fully interconnected network.

rejected at a significance level of $p<0.05$ after Bonferroni's correction for the number of connections.

3. Results

3.1. Motor cortex stimulation

Twenty five participants completed all three visits to provide motor cortex TMS/fMRI scans (25×3). The conventional analysis showed that motor cortex TMS induced activation M1, PMd, SMA, sensory cortex (parietal), auditory cortex (temporal) and thalamus.

3.1.1. DCM model comparison

Comparing four models of the four areas across all participants by BMS, the optimal model was found to be the first model. The alternative models we tested are shown in Fig. 1. The subject-specific Bayes factors for comparing the first model with the other three models and resulting group Bayes factors are reported in Supplemental Table 2. The model comparison among four models shows that Model 1 accounted better for the data than the alternative models (Supplemental Table 2). Therefore, this model (Fig. 1) was used to investigate connectivity for motor TMS data.

3.1.2. Intrinsic (task-independent) connectivity

Mean intrinsic connectivity parameters are displayed in Supplemental Table 3 and Fig. 2. Significant directional connectivities are the M1→PMd connection, the M1→SMA connection, the M1→thalamus and the PMd→SMA connection in placebo group. Note that the M1→PMd connection was also significant in both drug groups. To test our main hypothesis regarding the effect of anticonvulsant drugs versus placebo on the networks, we compared intrinsic connectivity parameters in LTG and VPA with respect to placebo. No significant difference was found between drug and placebo (see Fig. 2, Supplemental Table 3).

3.1.3. Drug modulatory effects of motor TMS

Fig. 3A shows motor TMS modulatory effect in the M1→PMd connection, the M1→SMA connection and the PMd→SMA connection in placebo group (Supplemental Table 3). To test our main hypothesis regarding the effect of the anticonvulsant drugs versus placebo on the networks, we compared modulatory effects of LTG and VPA with respect to placebo. The results showed that both LTG and VPA significantly reduced modulatory effect from M1 to PMd and M1 to SMA (Fig. 3D and E, Supplemental Table 3).

3.2. Prefrontal cortex stimulation

Twenty-one participants provided usable data from the prefrontal interleaved TMS/fMRI visits (21×3). Previous conventional analysis showed that prefrontal cortex stimulation induced significant activation of several brain regions such as medial prefrontal cortex (mPFC), sensory cortex and auditory cortex.

3.2.1. DCM model comparison

Comparing three models of the three-area across all participants by BMS, the optimal model was found to be the second model. The alternative models we tested are shown in Fig. 4. Supplemental Table 5 (available subject-specific Bayes factors for comparing the models) shows the subject-specific Bayes factors for comparing Model 2 with other models and resulting group Bayes factors. The model comparison shows that model 2 accounted for the data more effectively than the alternative models (Supplemental Table 5). Therefore, this model

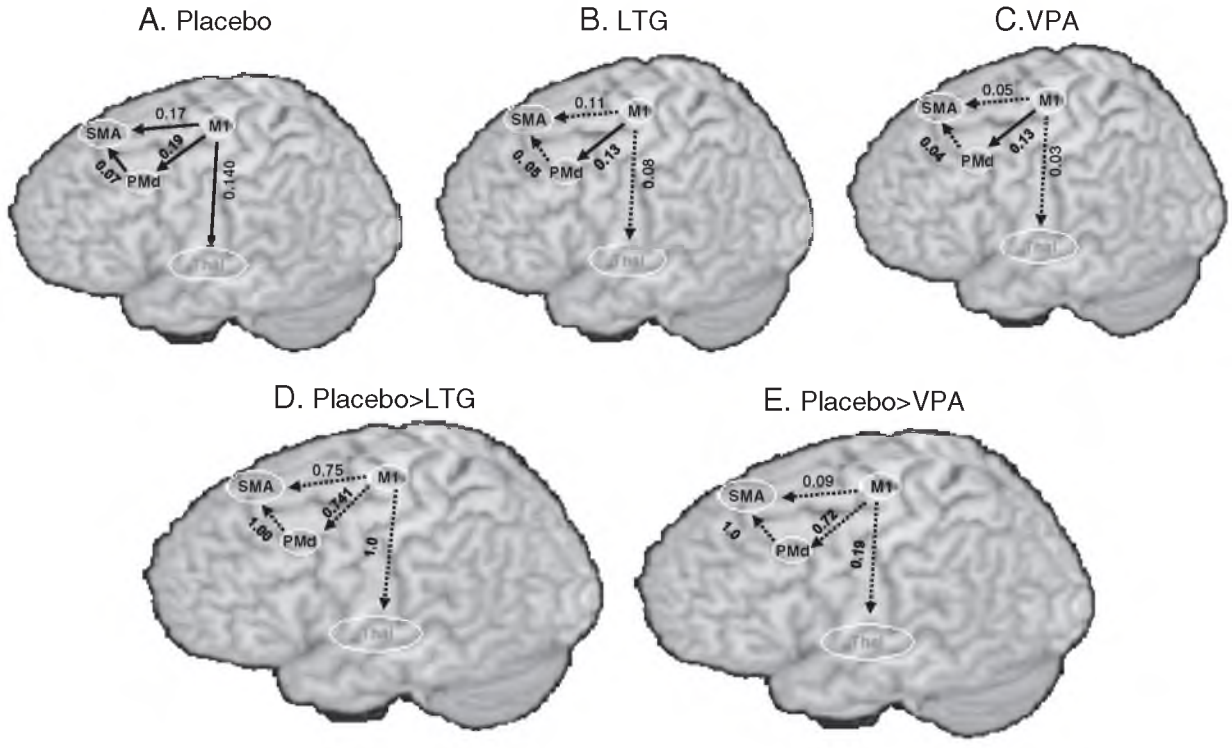

Fig. 2. Compared to placebo, LTG and VPA affect intrinsic connections related TMS (M1, PMd, SMA, thalamus, black labels indicate cortical region, gray labels indicate non-surface region). Intrinsic connections of Placebo (A), LTG (B) and VPA (C) in motor cortex are presented top pictures. The average strengths of influences across individuals are presented. Significant connections (posterior probability, >0.95, Bonferroni's correction) are presented in solid line and nonsignificant connections in dash line. Compared to placebo, LTG effect (D) and VPA effect (E) in intrinsic connections are presented in bottom pictures. Numerical values represent significant effect of drugs ($p<0.05$, Bonferroni's correction, labeled numbers are p value). The solid arrow indicates that drugs block the connectivity in motor circuit.

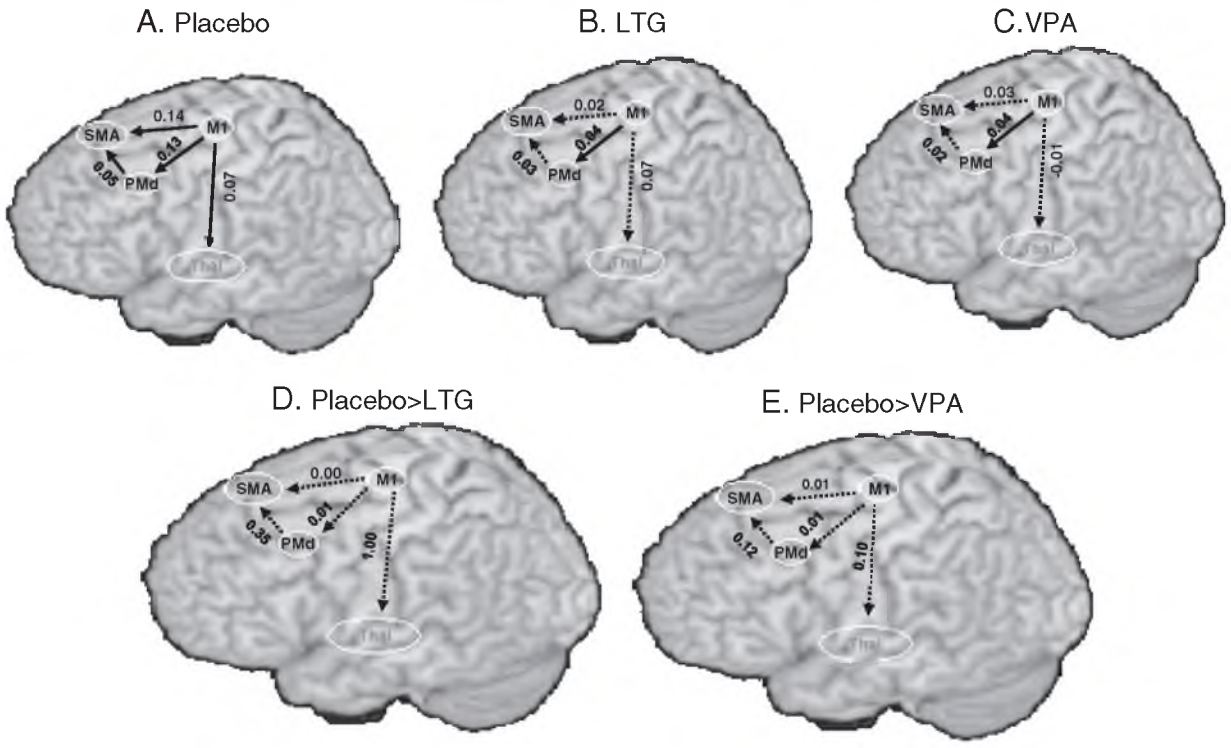

Fig. 3. Compared to placebo, LTG and VPA modulatory effects by TMS (M1, PMd, SMA, Thalamus, Black labels indicate cortical region, Gray labels indicate non-surface region). Modulations of Placebo (A), LTG (B) and VPA (C) in motor cortex are presented top pictures. The average strengths of influences across individuals are presented. Significant modulations (posterior probability, >0.95, Bonferroni's correction) are presented in solid line and nonsignificant connections in dash line. Compared to placebo, LTG effect (D) and VPA effect (E) in the modulation of TMS are presented in bottom pictures. Numerical values represent significant effect of drugs ($p<0.05$, Bonferroni's correction, labeled numbers are p value). The solid arrow indicates that drugs block the connectivity in motor circuit.

(Fig. 4) was used to investigate effective connectivity for prefrontal TMS data.

3.2.2. Intrinsic (task-independent) connectivity

Mean intrinsic connectivity parameters for suprathreshold TMS over DLPFC with drugs or placebo are displayed in Supplemental Table 6. Significant directional connectivity of the DLPFC → ACC, DLPFC → mPFC, mPFC → ACC and ACC → mPFC was only found during the session when participants took LTG.

3.2.3. Drug effect on intrinsic connectivity

To test our main hypothesis regarding the effect of anticonvulsant drugs on the networks, we found that LTG significantly increased connectivity from DLPFC to ACC (Fig. 5, Supplemental Table 6).

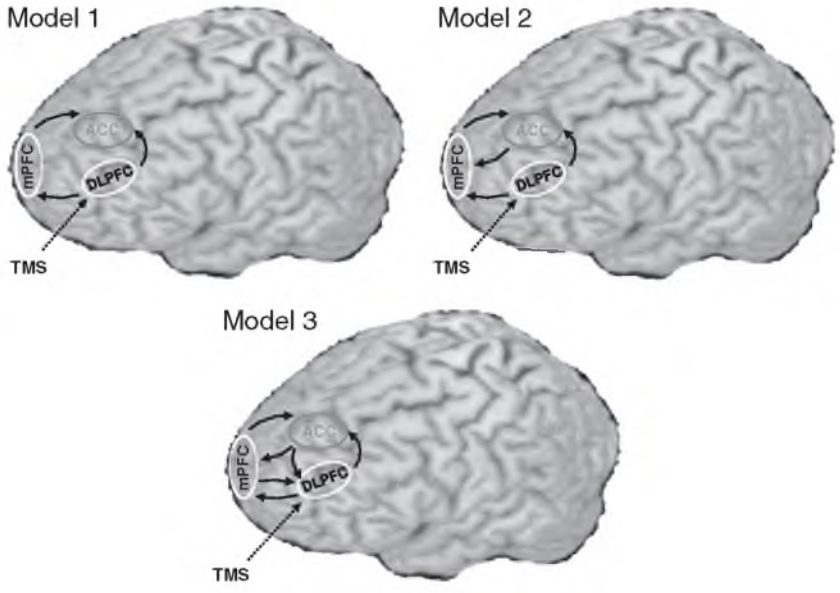

Fig. 4. Model network of interregional connections and experimental inputs for prefrontal cortex experiment. Regions were connected with stimulus inputs entering DLPFC, mPFC and ACC. The arrows and arrowhead highlight defining organizational features for each network model: Model 1, DLPFC outputs to both mPFC and ACC, ACC input from mPFC; Model 2, DLPFC outputs to both mPFC and ACC, mPFC and ACC bidirectionally connected; Model 3 is a fully interconnected network.

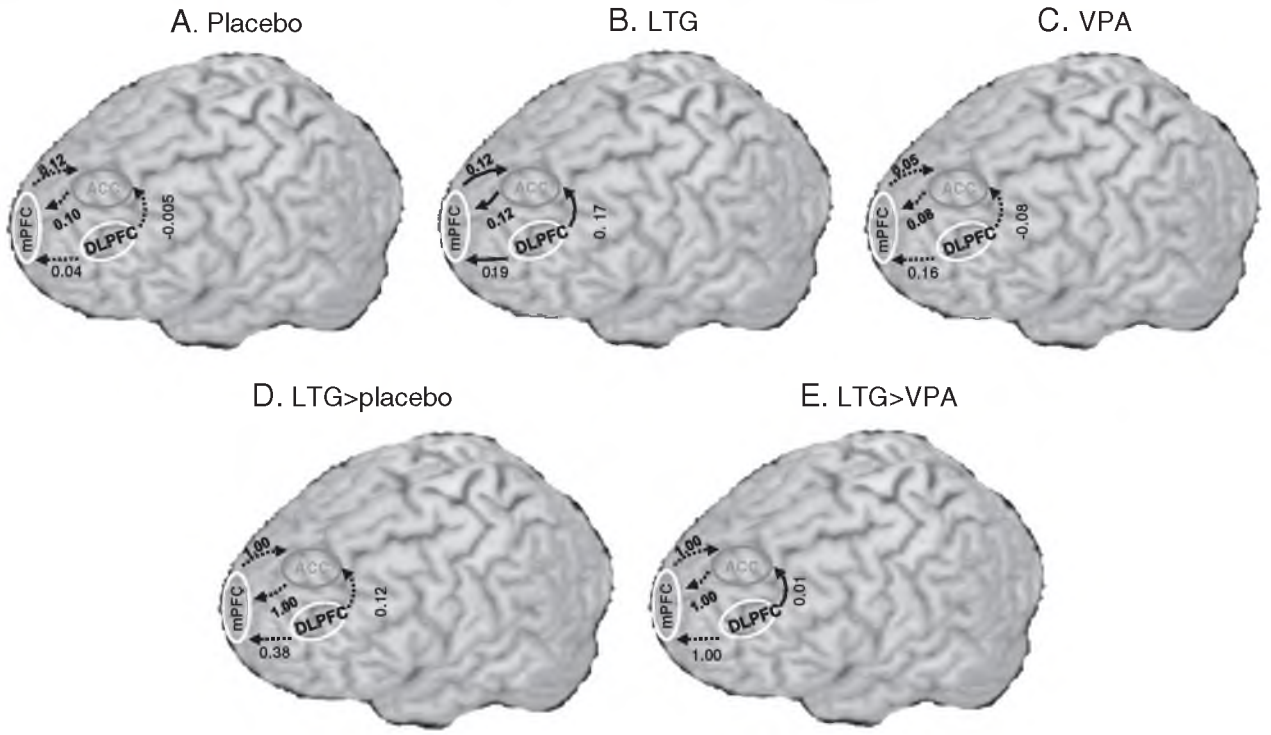

Fig. 5. Compared to placebo, LTG and VPA affect Intrinsic connections related TMS over DLPFC (DLPFC, mPFC, ACC. Gray indicates non-surface region). Intrinsic connections of Placebo (A), LTG (B) and VPA (C) in frontal-subcortical neuronal circuits are presented top pictures. The average strengths of influences across individuals are presented. Significant connections (posterior probability, >0.95, Bonferroni's correction) are presented in solid line and nonsignificant connections in dash line. Compared to placebo, LTG effect (D) and the differences between LTG and VPA (E) in intrinsic connections are presented in bottom pictures. Numerical values represent significant effect of drugs ($p<0.05$, Bonferroni's correction, labeled numbers are p value). The solid arrow indicates that drugs boost the connectivity in mPFC to ACC connections.

3.2.4. Drug effects on modulation of prefrontal TMS

Although we found a significant modulatory effect from mPFC to ACC, no drug effect was found in modulatory effects (Supplemental Table 6).

4. Discussion

To our knowledge, this is the first interleaved TMS/fMRI imaging study to directly compare the effects of different anticonvulsant drugs on motor or prefrontal neural circuits in the same participants. Our data suggest that LTG and VPA have similar effects on motor circuits but different effects on limbic circuits. LTG and VPA both significantly reduced modulatory effects in the M1 → PMd and M1 → SMA, but only LTG significantly increased directional connectivity of the DLPFC → ACC. These results may partially explain why LTG and VPA have similar efficacy for epilepsy yet different efficacy for bipolar disorder (Ketter et al., 2003; Moeller et al., 2009).

4.1. Effects of TMS on brain circuits

Task-dependent over-activation within several motor areas is a consistent finding in TMS studies (Lee et al., 2003; Li et al., 2004b; Bestmann et al., 2005). TMS-induced movement has been shown to cause BOLD changes in cortical regions such as SMA, PMd, and visual cortex(Grefkes et al., 2008). Additionally, the sound of the TMS coil and the sensation of its discharge on the scalp have been shown to induce BOLD changes in auditory and sensory cortices, respectively (Bohning et al., 1999; Bestmann et al., 2003). With conventional image analysis, we found that TMS produced brain activity in broad regions (Li et al., 2004a,b, 2010; Sack et al., 2007; de Graaf et al., 2009).

The analysis of task-independent (intrinsic) connectivity results supports previous data that show TMS affecting brain regions beyond the focal stimulation site (Bohning et al., 1999; Nahas et al., 2001; Bestmann et al., 2004; Li et al., 2004b; Sack et al., 2007; de Graaf et al., 2009). Among the four brain regions analyzed in this study, a significant connectivity was observed in the M1 → PMd connection, the M1 → SMA connection, the M1 → thalamus in connection and the PMd → SMA connection. Anatomically, the SMAs in each hemisphere are reciprocally connected and each projects to both contralateral and ipsilateral M1. Under normal conditions, however, activation of both SMAs may appropriately modulate M1 activity. In other words, activation of both SMAs may mediate the intended action and suppress unintended mirror movements (Muakkassa and Strick, 1979; Kasess et al., 2008). Similarly, the present study shows that TMS over M1 can modulate the M1 → PMd connection, the M1 → SMA connection and the PMd → SMA connection. We suggest that TMS induces activation in remote regions through efferent connections from the regions underneath TMS coil.

4.2. LTG and VPA inhibit motor circuits

The current data imply that both LTG and VPA alter network connectivity between several motor areas, including M1, PMd, and SMA. The two drugs showed similar significant inhibition of motor circuits including connections from M1 to PMd and M1 to SMA. The current data imply that both LTG and VPA produce a similar anticonvulsant mechanism that blocks motor circuit pathways from M1 to PMd and M1 to SMA. With respect to the mechanisms of action researchers have proposed that anticonvulsant drugs be divided into three classes based on their ability to block sustained high-frequency repetitive firing of action potentials: (1) those that block voltage-dependent Na^+ channels, (2) those that enhance GABAergic inhibition, or (3) those that block slow, pacemaker-driven, repetitive firing and T-Ca^{2+} current (Loscher, 1998).

LTG binds and stabilizes the inactivated state of voltage-gated sodium channels, thus limiting sustained repetitive neuronal activity

without substantially affecting normal synaptic activity (Xie et al., 1995). Previous combined TMS/fMRI studies have shown that LTG can reduce activation of local motor cortex by TMS as well as inhibit cortical excitability (Li et al., 2004b, 2010). The present network analysis showed that LTG also reduced activation of motor cortical circuits which include the modulatory effect in the M1→PMd connection, the M1→SMA connection. Interestingly, VPA has a similar effect in motor circuits without any effect on cortical excitability (Li et al., 2009).

4.3. LTG and VPA differentially affect corticolimbic circuits

In a recent study, fronto-cingulate effective connectivity in both depressed and healthy individuals was analyzed with DCM. The results showed that intrinsic connections between prefrontal cortex and dorsal ACC were significantly different in depressed individuals than they were in healthy controls (Schlosser et al., 2008). Our hypothesis was that LTG and VPA would have different effects on DLPFC and related circuits, and that these differences might underlie their different clinical profiles in the treatment of bipolar depression (Ketter and Calabrese, 2002; Gajwani et al., 2005). The current network analysis showed that ACC was significantly connected to the cortical area (DLPFC) underneath the TMS coil when participants took LTG compared to placebo. This connection was not evident in participants after VPA compared to placebo. Most importantly, our results showed that LTG increased the DLPFC→ACC connection compared to VPA (Fig. 5). These results demonstrate that LTG and VPA have different effects on corticolimbic circuits. One could speculate that these differences might underlie the different clinical profiles of LTG and VPA in the treatment of bipolar depression (Ketter and Calabrese, 2002; Gajwani et al., 2005).

The present results are also consistent with a recent imaging study by Haldane et al. (2008) in which fMRI was used to investigate the effect of LTG on the neural circuits underlying working memory and emotional processing in 12 patients with bipolar disorder. In this study, LTG monotherapy over 12 weeks was associated with increased task-dependent activation within the prefrontal cortex and cingulate gyrus. The authors suggest that LTG may enhance cortical function within neural circuits involved in memory and emotional self-regulation (Haldane et al., 2008).

4.4. Combined TMS and fMRI in CNS drug development

Drug development needs to balance agility, speed and risk in defining the probability of success for molecules, mechanisms and therapeutic concepts. Over the past decade, researchers have used TMS measures of cortical excitability in CNS-active drug studies, especially in anticonvulsant drugs (Boroojerdi, 2002; Ziemann, 2004). Since these measures are based on the amplitude of MEP, the information concerning the drug effects is limited to motor cortex. Hence, finding methods of measuring drug effects in other non-motor brain regions is important. Functional MRI has provided another method to examine drug effects in the brain, referred to as phMRI (Borsook et al., 2006). However, there are several problems associated with the activation paradigms in conventional phMRI (e.g. different behavioral response from healthy participants to patients) (Wise and Tracey, 2006). Combining TMS with functional MRI may provide a bridge connecting motor cortex excitability measures to BOLD functional response. Furthermore, combining functional MRI and DCM analysis may allow for network analysis of medication effects.

4.5. Limitations

A number of limitations to the current study could be addressed in future studies. First, the methods for coil placement over frontal cortex were based on a probabilistic method used previously (George et al., 1995). This crude algorithm likely resulted in a spread of activation as documented by others (Herwig et al., 2001). In future studies, T1 structural or even activation based MRI scans to locate the prefrontal stimulation site may be used to reduce inter-subject variability.

Second, the regions selected for DCM analysis were based on those identified in previous studies with TMS/fMRI, as well as conventional fMRI results from the current study (Lee et al., 2003; Li et al., 2004b, 2010). We excluded auditory and sensory cortical regions from the DCM analysis because we assumed that these regions were most likely activated by auditory or sensory stimuli secondary to the TMS. Since interpretations of studies using DCM only pertain to those regions and connections specified in the chosen model, we cannot rule out the contribution of other regions to our regions of interest.

Third, we limited our analysis to three regions within the prefrontal cortex. While this approach enabled us to analyze data from more individuals, it limits our ability to make conclusions about additional regions of the prefrontal cortex and their corticofugal projections.

Finally, we cannot claim that all changes in effective connectivity observed after LTG or VPA would appear similarly in epilepsy and mood disorders. Further investigation using the interleaved fMRI/TMS technique in patients with epilepsy and mood disorder would be valuable.

4.6. Conclusions

Using the combined TMS-fMRI technique in 30 healthy men, DCM analysis showed that both LTG and VPA significantly reduced connectivity between M1 and PMd and between M1 and SMA when TMS was applied over motor cortex. These results suggest that anticonvulsant drugs may produce their therapeutic effect by reducing excitability within motor circuits and within motor cortex. Consistent with the positive effect of LTG on activation of cortico-limbic structures following TMS applied over prefrontal cortex, LTG alone also increased effective connectivity between cortical regions underneath the TMS coil and the ACC. These results demonstrate for the first time that the combination of TMS/fMRI can be used to investigate how drugs affect neural circuits relevant to different neurological or psychiatric disorders. The technique promises to be valuable in predicting and understanding the clinical efficacy of novel and even existing CNS-active drugs.

Supplementary materials related to this article can be found online at doi:10.1016/j.pscychresns.2011.04.012.

Acknowledgments

This study was funded primarily by an unrestricted research grant from GlaxoSmithKline to Dr. George, as well as from Center for Advanced Imaging Research and Brain Stimulation Laboratory infrastructure and resources. CHL is a full-time employee of GlaxoSmithKline S.p.A. None of the other authors has equity or financial conflicts. Drs. Li and George had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Presented in abstract form at the annual meeting of American College of Neuropsychopharmacology, Hollywood, FL, Dec 14, 2006 and the annual meeting of the Society of Biological Psychiatry, San Diego, CA, May 21, 2007.

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