Altered Prefrontal Excitation/Inhibition Balance and Prefrontal Output: Markers of Aging in Human Memory Networks

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Abstract

Memory impairments and heightened prefrontal cortical (PFC) activity are hallmarks of cognitive and neurobiological human aging. While structural integrity of PFC gray matter and interregional white matter tracts are thought to impact memory processing, the balance of neurotransmitters within the PFC itself is less well understood. We used fMRI to establish whole-brain networks involved in a memory encoding task and dynamic causal models (DCMs) for fMRI to determine the causal relationships between these areas. These data revealed enhanced connectivity from PFC to medial temporal cortex that negatively correlated with recall ability. To better understand the intrinsic activity within the PFC, DCM for EEG was employed after continuous theta burst transcranial magnetic stimulation (TMS) to the PFC to assess the effect on excitatory/inhibitory (E/I) synaptic ratios and behavior. These data revealed that the young cohort had a stable E/I ratio that was unaffected by the TMS intervention, while the aged cohort exhibited lower E/I ratios driven by a greater intrinsic inhibitory tone. TMS to the aged cohort resulted in decreased intrinsic inhibition and a decrement in memory performance. These results demonstrate increased top-down influence of PFC upon medial temporal lobe in healthy aging that is associated with decreased memory and may be due to unstable local inhibitory tone within the PFC.

Key words: aging, dynamic causal modeling, EEG, fMRI, memory

Introduction

Memory impairments that develop as a result of aging in the absence of pathologies have been attributed to changes in 3 neurobiological factors including gray matter volume (Rodrique and Raz 2004), white matter integrity (Damoiseaux et al. 2009; Metzler-Baddeley et al. 2011), and interregional functional connectivity (Grady et al. 2003; Wang et al. 2010; Mormino et al. 2011; Nakagawa et al. 2013), with evidence for independent contributions from each of these dimensions (He et al. 2012). Many of these findings have been found in the prefrontal cortex (Salat et al. 2004; Persson et al. 2006), which is recruited during successful memory encoding into a network comprising medial temporal and posterior parietal brain regions (Dolan and Fletcher 1997). Neuroimaging studies of aging cognition repeatedly demonstrate a shift to increased and less lateralized patterns of activation in prefrontal cortex (PFC; Cabeza et al. 1997; Morcom et al. 2003; Davis et al. 2008; Morcom and Friston 2012), though it is uncertain whether heightened prefrontal activity indicates...
pro-performance compensatory signaling or is indicative of cognitive decline (Rajah and D’Esposito 2005; Grady 2008).

In terms of prefrontal neurotransmitters, age-related assessments of human prefrontal neurochemical change have largely implicated reduced dopaminergic neurotransmission in affecting cognitive decline (Backman et al. 2006), though methodological limitations have impeded a fuller decomposition of task-related neurotransmission. While in non-human primates, recent analysis of in vivo electrophysiological recordings in the PFC has shown that maturation is associated with a decline in intrinsic inhibitory connectivity (Zhou et al. 2014). Morphological assessments of prefrontal networks and excitatory/inhibitory (i.e., circuitry that does not directly synapse on regions outside the local PFC neighborhood) in neurological disorders (Boly et al. 2011; Marreiros et al. 2013). We hypothesized that aging effects on both long-range and local intrinsic prefrontal physiology play a role in age-related memory decline.

Here, we applied both DCM for fMRI and DCM for electroencephalography (EEG) to characterize age-effects on whole-brain networks and excitatory/inhibitory (E/I) synaptic balance within the PFC, respectively. We were interested in whether these measures were associated with encoding and subsequent recall in a paired association memory task (Haskins et al. 2008). Utilizing DfMRI ensured that a whole-brain assessment of performance-related connectivity would be accessible, allowing for the dissociation between interregional effects and intrinsic within-region connectivity. DCM for EEG uses canonical neural mass models and established synaptic kinetics that allow for a focused assessment of inhibitory and excitatory synaptic circuitry within the PFC. In addition, we applied continuous theta burst (cTBS) transcranial magnetic stimulation (TMS) to the PFC to assess the effect on local synaptic physiology in both the young and aged cohorts. cTBS is a TMS protocol (Huang et al. 2009) that affects GABAergic interneuronal circuits in motor cortex (Stagg et al. 2009; Di Lazzaro et al. 2012), and thus, it is hypothesized that this intervention will preferentially affect the inhibitory connections within the prefrontal cortex and further that this will have a behavioral effect on memory performance by disrupting these GABAergic circuits. Our overall goal was to elucidate the cellular and synaptic source of age-related intrinsic connectivity deficits identified in the PFC that may contribute to whole-brain functional connectivity and behavioral impairments.

Materials and Methods

Experiment 1 (fMRI)

Participants

Two groups of participants, 15 older adults (mean = 63 years, range 54–75, 9 females) and 14 younger adults (mean = 26 years, range 19–38, 8 females), partook in the fMRI experiment. Participants were screened for any diagnosis or history of psychiatric and/or neurological disorders and MRI contraindications; all were fluent in English. Study protocols were approved by the Virginia Tech Institutional Review Board and written informed consent was obtained from each participant. Participants were compensated for their participation. Each participant had normal or corrected-to-normal vision and all passed a visual color assessment.

Experimental Protocol

Encoding phase. While in the MR scanner, participants were presented with 28 pairs of images of common household objects (Fig. 1). These image pairs were presented for 3000 ms and were presented within a block. Blocks were defined by 1 of 2 stimulus–attribute questions (Morcom et al. 2010) “Anything Red?” (detail orientation) or “In a kitchen?” (context orientation), to which participants indicated their response with a yes/no button press. Trials were jittered with an interstimulus interval of 1000 ± 500 ms, during which time a fixation cross appeared onscreen (Fig. 1A). A block order was counterbalanced across subjects. Fourteen pairs were shown at random within the context-oriented blocks, and 14 different image pairs were presented within the detail-oriented blocks. Each pair was presented 10 times (296 trials; Fig. 1A). Participants were given a limited time (3000 ms) to answer the questions, while the images remained on screen and were instructed to focus on answering the questions as quickly and accurately as possible. Prior to formal testing, participants were informed that item pair recall would be tested after the scan.

Recall phase. Participants then waited for 1 h before completing the recall task. Only behavioral data were collected during the recall task. Participants were seated in a quiet computer booth and presented with 56 pairs (28 correct and 28 mismatched pairs) and asked to correctly identify images which were paired together during the encoding phase and to identify image pairs that did not appear together during encoding with a yes/no button press. Participants were also asked about their certainty in their response for each pair. Responses were self-timed.

fMRI Data Acquisition

Images were collected using a 3-T Siemens MAGNETOM Trio scanner. High-resolution $T_1$-weighted structural images were collected using an MPRAGE sequence with a repetition time (TR) = 1200 ms, echo time (TE) = 2.66 ms, field of view (FOV) = 245 mm, and 1.0 mm slice thickness. Echo-planar image (EPI) data were acquired with a TR of 2000 ms, TE 25 ms, FOV 220 mm, with 37 interleaved slices acquired at a slice thickness of 4.0 mm. Slices were oriented 30° superior-caudal to the plane through the anterior and posterior commissures to reduce signal dropout. Headphones were used to reduce scanner noise. Participants used a mirror to view the stimuli projected behind them in the scanner.

fMRI Data Analysis

Preprocessing and data analysis were performed using the statistical parametric mapping software implemented in Matlab (SPM12b beta; http://www.fil.ion.ucl.ac.uk/spm). The first 6 images of the acquisition were discarded to allow for equilibrium magnetization. EPI blood oxygen level-dependent (BOLD) images were realigned and resliced using a six parameter spatial transformation with the first nondiscarded scan as the reference (estimated motion parameters were used as nuisance regressors in the first-level general linear model, GLM). The mean resliced image was also computed and the structural image was co-registered to this mean image. The unified segmentation routine was then used to perform segmentation bias correction and spatial normalization. Images were normalized to the MNI space using...
the ICBM template. Finally, the data were smoothed using a kernel with 6-mm full-width at half maximum.

Individual BOLD responses were analyzed using a GLM. Four regressors of interest were entered into the GLM; these were delta functions representing event-based image onsets with parametric modulators representing reaction times for each trial and 2 block regressors identifying the onset of both stimulus-attribute blocks. These responses were modeled using a boxcar function of 16 s duration. All regressors were convolved with a canonical hemodynamic response function.

We used a summary statistic approach to assess group-level whole-brain activations related to encoding and stimulus-attribute attention. Specifically, we computed F-contrast images for each individual subject’s response to the onset of the stimulus-attribute blocks.
pairs (Onsets /< 0) as well as contrasts for each specific block type (Kitchen/< 0) and (Red /< 0). At the group level, we tested for temporal cortical areas that responded positively or negatively to image onset using activations corrected at the whole-brain level using a family-wise error (FWE) rate of P < 0.05 within a region-of-interest mask that included bilateral hippocampal, parahippocampal, and temporal pole regions. We also assessed encoding context and attribute processing using an F-test over the 2 block-based contrasts. Activations to stimulus-attributes were assessed using whole-brain activity with peak activations deemed significant at P < 0.05 FWE-corrected (Supplementary Table 1).

**Dynamic Causal Modeling for fMRI**

Having identified regions that responded to image presentation [right hippocampus (RHPC) and visual association area (VAA)] and stimulus-attribute decisions [right inferior frontal gyrus (RIFG)] at the group level, we then extracted BOLD time series from each participant’s fMRI data individually. Time series were extracted from RHPC and VAA using a T-contrast over “Onsets” with a liberal P-value threshold of P < 0.1 (note: P-values here are used to define the voxel cluster from which the principal eigenvariate will be extracted, they are not involved in the final DCM statistics). Time series were extracted from RIFG using an F-contrast over “red” and “kitchen” with a P-value threshold of P < 0.1. Voxels of interest were identified around the group peak coordinates (VAA: [0, −90, 28]; RHPC: [22, −30, −4]; RIFG: [48, 24, −6]), and the principle eigenvariate in a sphere of 6 mm was extracted for the model analysis (individual peaks are summarized in Supplementary Table 2). Time series were corrected for effects of interest to incorporate effects from the 3 regressors: “onsets,” “red,” and “kitchen.”

To test the effective connections across the memory network, we constructed 3 models of potential interactions among our 3 volumes of interest. These models are shown in Figure 28. The difference between these models is based on the modulatory effects of the stimulus-attribute block. All the models have onsets originating at the VAA with bidirectional connections among all 3 nodal models. All nodal models also have detail or context orientation block inputs to the IFG and VAA. In the first model, contextual modulation is present in the top-down connections from IFG to VAA and from IFG to HPC. The second model prescribes bidirectional modulation of contextual control from VAA to IFG and from HPC to IFG. The third model has bidirectional modulatory connections.

Bayesian Model Selection was used to compare these 3 competing architectures using a fixed-effects analysis, and checked for individual consistencies (Stephan et al. 2009). Finally, connectivity parameters were extracted from each individual’s DCM and correlated with recall performance.

**Behavioral Analysis**

Participants’ recall ability of image pairs 1 h post encoding was quantified as the percentage of red and percentage of kitchen pairs accurately judged as being seen or not during encoding. For example, a correct response was a “yes” to a seen pair or a “no” to an unseen pair during the encoding phase. A total of 28 pairs of each were presented during recall, and participants’ recall ability was calculated for red and kitchen separately (number of correct responses/28) x 100. This number was calculated for each participant and a two-way analysis of variance (ANOVA) with factors Age (young, old) and Item [red, kitchen], was performed. To assess the relationship of recall ability with functional connectivity, Pearson correlation coefficients were derived for both the IFG to VAA connection and the IFG to medial temporal cortex.

**Experiment 2 (EEG–TMS)**

**Participants**

Two groups of participants, 24 older [mean = 69.0 years, range 65–78, 6 females, Mini-Mental State Examination (MMSE) = 29.5, range 27–30] and 30 younger adults [mean = 23.6 years, range 18–30, 17 females, MMSE = 29.9, range 29–30], voluntarily participated in the study. All participants were fluent in English, screened for psychiatric and neurological diagnoses and TMS contraindications, and screened for cognitive impairment using the MMSE. Study protocols were approved by the Virginia Tech Institutional Review Board and written informed consent was obtained from each participant. Participants were compensated for their participation. Each participant had normal or corrected-tonormal vision and had passed a visual color assessment.

**Experimental Protocol**

Participants were seated in a magnetically shielded room in a high-back chair and viewed visual stimuli on a computer monitor positioned on a table in front of them. The details of the encoding phase presentation are identical to those of Experiment 1, except that participants recorded their response on a keyboard positioned on a table in front of them. The recall phase was identical to Experiment 1.

**Electroencephalography**

During the encoding phase, EEG data were acquired using a DC amplifier (BrainAmp MR Plus, Brain Products GmbH, Gilching, Germany) with an ActiCap 64 electrode cap (Brain Products, EASYCAP GmbH). Electrodes were prepared with Silvadex gel (Brain Products GmbH) and electrode impedances were verified <5 kΩ prior to formal data collection. EEG data were sampled at 1000 Hz and filtered online at DC-250 Hz before being stored on a computer for later analysis. During TMS, electrodes over RIFG were removed to accommodate the TMS coil. After stimulation, electrodes were replaced and impedance verified.

**Transcranial Magnetic Stimulation**

Approximately 5 min prior to the encoding task, participants underwent cTBS (young: N = 14, old: N = 12) or a sham (young: N = 16, old: N = 12) stimulation condition, with participants assigned from the young and old group at random. cTBS was performed using an MagPro X100 Stimulator (MagVenture, Inc., Atlanta, GA, USA) and Cool-B65-A-P-Butterfly-Coil (2 layers of 5 windings at each wing, winding height 12 mm, inner diameter 35 mm, and outer diameter 75 mm). The coil was positioned between the international 10–20 electrode sites F6 and F8, with the coil handle angled 45° outward from the midline. The protocol was designed to target and modulate the RIFG corresponding to MNI coordinates (48, 24, -6) informed by our fMRI results. This coil position was determined using a finite element method model built using SimNibs as previously described (www. simnibs.org; Windhoff et al. 2013). The cTBS repetitive protocol was set to a 20% maximum stimulator for all subjects to avoid stimulation of the superficial nerves and musculature of the face and eye. This amplitude is in a range that has been demonstrated to have a physiological effect (Opitz et al. 2015). Participants received 40 s of cTBS according to Huang et al. (2005). Briefly, this consisted of 3 single biphasic bursts separated by 0.02 s (50 Hz) repeated every 0.2 s (5 Hz). A total of 600 pulses were delivered in 40 s. Participants were given earplugs to reduce
the noise of the cTBS. For sham stimulation, participants were also given earplugs and a nonfunctional biologically inert weighted object; the size and shape of the TMS coil (height 18 cm, width 8 cm) was used in place of the cTBS coil as per recommendations of Opitz et al. (2015). Auditory stimulation was similar as the TMS unit was on in the vicinity of their head. To ensure proper shamming, participants were unable to view the coil or the stimulation procedure.

EEG Data Analysis
EEG data analysis was performed using custom scripts and EEGLAB v13.3.2b (Delorme and Makeig 2004), Statistical Parametric Mapping for EEG (v.12b) (http://www.fil.ion.ucl.ac.uk/spm/), and Matlab v7.10.0 (The Mathworks, Inc., Natick, MA, USA). EEG data were down-sampled to 250 Hz and band-pass filtered (1–60 Hz) using a Hamming windowed finite impulse response filter. Data were then re-referenced to the average of 64 channels and epoched (−400 to 1500 ms) around image onset. EEG data were manually checked for artifact and eye blinks removed as well as using an automatic channel peak-to-peak threshold of 120 µV. Time epochs that contained artifact was removed from subsequent analysis.

Gamma Activity in the Prefrontal Cortex
We concentrated on gamma activity as increases and decreases in the power of this frequency band of the local field potential (LFP) reliably follow increases and decreases in the BOLD signal (Magri et al. 2012). Sourcing of oscillatory gamma activity in the right and left PFC was performed using the Linearly Constrained Minimum Variance (LCMV) beamforming method in the DAiSS

Figure 2. Memory network and models. (A) Group-level (N = 29) sources for fMRI DCMs displayed at P < 0.001 uncorrected. VAA (peak voxel, P < 0.05 whole-brain FWE-corrected at: [0, −90, 28] MNI, F = 19.64) and temporal–cortical sources in RHP2 responded to image–pair presentation (peak voxel P < 0.05 whole-brain FWE-corrected at: [22, −30, −6] MNI, F = 69.12), while block-based regressors were correlated with BOLD in RIFG (peak voxel, P < 0.05 whole-brain FWE-corrected at: [48, 24, −6] MNI, F = 18.00). (B) Three DCMs were proposed to account for BOLD dynamics during encoding: Model 1 expressed stimulus–attribute modulations along top-down connections only, Model 2 expressed attribute modulations along the corresponding bottom-up connections only, and Model 3 expressed both top-down and bottom-up context control mechanisms. (C) Group (N = 29) Bayesian fixed-effects analysis of the performance of the models. Model 1 was the optimal model with a group log Bayes Factor (InGBF) of 5248 when compared with Model 3 (the second best performing model).
Using the time series extracted from left and right prefrontal cortex, we optimized a DCM of cross-spectral densities (Friston et al. 2012). These data were then used for DCM analysis. DCMs for EEG, Cross-Spectral Densities

Using the time series extracted from left and right prefrontal cortex, we optimized a DCM of cross-spectral densities (Friston et al. 2012) for each participant. We used gamma-band activity (30–55 Hz) from 60 to 250 ms and inverted (“fit”) an LFP model of 2 interacting sources (left PFC and right PFC), which were connected via lateral connections (see Supplementary Fig. 1). These models are imbued with dynamics of synapses at 3 distinct cell subpopulations within each source: glutamatergic layer IV spiny stellate cells, supra- and infragranular pyramidal cells, and GABAergic inhibitory interneurons. These neuronal ensembles are connected as per Figure 5A. Dynamics are parameterized with postsynaptic depolarization and hyperpolarization kinetics according to the type of afferent neurotransmitter and populations are modeled to fire at an ensemble average rate according to the mass postsynaptic potential (Supplementary Fig. 1). Crucially within each prefrontal source, the strength of afferents is individually parameterized ($t_{1,5}$, Fig. 5A), allowing us to test the effects of age and TMS on GABAergic and glutamatergic connections separately (where 2 composite intrinsic connectivity measures were analyzed comprising net inhibitory and net excitatory connections within the PFC). Models were fit to the full complex cross-spectrum and were initialized using standard SPM parameter priors. Crucially, the neural mass models contain parameters that control the strength of intrinsic dynamics among cell types within a region. In all, 5 intrinsic connectivity parameters were optimized for each source ($t_{1,5}$, Fig. 5A). These parameters of the DCM are log-scaling values (David et al. 2006), which positively scale prior intrinsic connectivity strength ($F$-test to test the hypothesis that cTBS in the aged cohort reduced memory accuracy.

Behavioral Analysis

Participants’ recall ability 1 h post encoding was quantified as for the MRI experiment. These data were subjected to a three-way ANOVA with factors TMS [real, sham], Age [young, old], and Item [red, kitchen]. Examination of significant interactions was performed with suitable post hoc testing. In a follow-up study to better test the effect of the TMS intervention, we had 21 of the 24 original aged EEG participants to perform the identical memory task under the other TMS condition (TMS/Sham) to be able to satisfy a repeated-measures design. Participants’ recall ability was quantified as above and these data were subjected to a one-tailed paired t-test to test the hypothesis that cTBS in the aged cohort reduced memory accuracy.

Results

fMRI-Based Networks

Participants were presented with pairs of images and told to retain the pair for a subsequent memory test. Images were presented in a mixed, event-related, block design with individual pairs shown randomly, within 1 of 2 blocks associated with a particular stimulus-attribute decision task (Morcom et al. 2010), where participants made yes/no judgments about item color (“are the items colored red?”) or context (“are the items found in a kitchen?”; Fig. 1A). In the subsequent test of memory, older participants were significantly worse at recall ability ($P \leq 0.0001$), though there was no main effect of the type of item presented ($P = 0.72$), nor an interaction (Fig. 1B). To identify temporocortical sources within the network subtyping encoding, we analyzed event-related responses to image presentation. Applying an inclusive anatomical mask of bilateral parahippocampus, hippocampus, and temporal poles over whole-brain activity, we found significant responses in the right posterior HPC (peak voxel, $P < 0.05$ whole-brain FWE-corrected at $[22, −30, −4]$ MNI coordinates, $F = 69.12$; see Supplementary Table 1 and Fig. 2A). To assess stimulus-attribute processing, we analyzed whole-brain activations that responded to block-based activity during either detail or context-based blocks. We found significant activation of VAA (peak voxel, $P < 0.05$ whole-brain FWE-corrected at $[0, −90, 28]$, $F = 19.64$) and of RIFG (peak voxel, $P < 0.05$ whole-brain FWE-corrected at $[48, 24, −6]$, $F = 18.00$; Fig. 2A). To assess whether the VAA was a good candidate for the image-onset dynamic model input, we tested the image-onset contrast using an inclusive mask of the block-responsive contrast and revealed overlapping activation in area VAA at peak $[0, −90, 28]$. Thus, the image-onset contrast was used for eigenvalue extraction of VAA responses for the DCM (see Materials and Methods).

Having established the brain regions active during this task across the group, we then applied DCMs to each individuals’ BOLD responses. This effective connectivity analysis was performed after time series extraction for each participant around group-peaks in HPC, RIFG, and VAA in response to image pair presentation (RIFG and VAA) and block context (RIFG, Supplementary Table 2). Our models embodied 3 potential hypotheses about encoding and its prefrontal control: Model 1 represented a top-down processing model, where block-dependent responses
to stimulus attributes were driven by alterations in RIFG to VAA connectivity and RIFG to HPC connectivity (Fig. 2B). Model 2 represented a bottom-up mechanism where control processes instead were formed by changes in connectivity from VAA to RIFG and HPC to RIFG. Finally, Model 3 proposed that both top-down and bottom-up mechanisms were engaged. In addition, Models 1–3 comprised bidirectional endogenous connectivity among all 3 nodes with image-onset inputs to VAA and block inputs to VAA and RIFG (Fig. 2B). All connection strength measures were optimized based on individual BOLD responses (Stephan et al. 2010). Model inversion was performed using standard variational Bayesian approaches (Friston et al. 2003), and all models were linear and stochastic (Daunizeau et al. 2011).

Using a fixed-effects analysis, we performed a Bayesian model comparison to assess model-goodness over the group (Stephan et al. 2009). This test identified Model 1 as the winning model (Fig. 2C) whereby stimulus-attribute attention during encoding modulated only top-down connections from IFG to VAA and from IFG to HPC. This model had a log Bayes Factor (Stephan et al. 2009) of 5248 (Baas and Raftery 1995), representing very strong evidence in favor of Model 1 compared with Model 3—the reciprocal model which was the second best performing model (Fig. 2C). Though fixed-effects analysis can be sensitive to outliers, we investigated winning models at an individual level and showed that 28 of the 29 participants had greater evidence in favor of Model 1, whereas one participant favored Model 2 (Supplementary Fig. 2). We then examined the connectivity parameters from both the aged and young groups for both the IFG to VAA and the IFG to HPC connections. The two-way separate groups ANOVA revealed a main effect of the connection parameter (P = 0.0014) and a significant interaction between age and the connections (P = 0.0058; Fig. 3A). Post hoc Tukey–Kramer comparisons revealed that the interaction was driven by significantly less modulation of the IFG to VAA for the old group (P < 0.05) and significantly greater modulation of the IFG to HPC for the old group (P < 0.05). We next examined the connectivity parameters from each individual’s DCM for a correlation with recall performance. We found that the connection strength from IFG to HPC was significantly negatively related to recall performance (R = −0.51, P = 0.0047), but that there was no significant relationship between connectivity parameters from IFG to VAA (R = −0.10, P = 0.60; Fig. 3B). To probe what synaptic and cellular mechanisms within the prefrontal cortex may be mediating these connection differences, we employed DCM for EEG during the same task in a second cohort of young and aged participants.

EEG–TMS-Based Physiological Substrates

We used TMS in our second experiment in younger and older adults to manipulate prefrontal physiology and behavior. Specifically, the region of RIFG, identified as a mediator of disruptive hyperactive outputs in the fMRI experiment, was subject to either a cTBS protocol (Huang et al. 2005) or sham stimulation prior to task performance. This revealed prominent bilateral prefrontal activity, with peaks in right [22, 62, 4] frontal cortex (P < 0.05 FWE-corrected; Fig. 4A). The three-way ANOVA of behavior revealed a significant effect of age on recall ability (P ≤ 0.0001), and a significant interaction of age and TMS (P = 0.017). Tukey–Kramer post hoc examination of the interaction revealed that the interaction was driven by a significant difference between the aged and young cohort only for the cTBS condition (P < 0.05) where the young group accuracy increased and the aged group accuracy decreased.

From the prefrontal regions identified in the source-localization analysis, we extracted individual time series for the DCM analysis. The DCMs were optimized based on the complex cross-spectra from the left and right PFC from each subject individually (Friston et al. 2012). As evidenced from Figure 4C, this model was able to fit individual differences in gamma-band features. Figure 4C displays very different absolute cross-spectral density profiles across the gamma-band from the best performer and the worst performer. To ensure the low-intensity TMS used in this study stimulated the cortex, a finite element method computer model was conducted to simulate the electric field produced in the cortex from 20% maximum TMS machine output. As can be seen from Figure 4C, the location of the coil between EEG electrode positions F6–F8 produced a maximal electric field in the inferior frontal gyrus. We estimate the magnitude of the electric field strength produced by 20% maximum stimulator output to be in the range of 14–22 mV/mm. This field strength is approximately an order of magnitude higher than what is minimally needed to cause an effect on neurons (Francis et al. 2003).
ally higher inhibitory tone than the young cohort (Post hoc analysis revealed that the aged cohort has an intrinsic-
age and TMS (drove the main effect of age. Interestingly, TMS drove this tone

Figure 4. DCM for EEG reveals inhibitory deficit within the older PFC. (A) Group young and aged (N = 54) localized EEG gamma-band (30–55 Hz) activity during memory encoding. Prefrontal peaks included right superior frontal gyrus (peak voxel, \( P < 0.05 \) whole-brain FWE-corrected at: \([-22, 62, 4]\) MNI, \( T = 8.27 \)) and left superior frontal gyrus (peak voxel, \( P < 0.05 \) whole-brain FWE-corrected at: \([55, 22, 6]\) MNI, \( T = 9.26 \)). (B) DCM fits to source localized gamma-band activity in the PFC. Spectral responses (dashed lines) and model fits (filled lines) for the best performing younger participant (top) and the worst performing older participant (bottom). The DCMs accurately captured the gamma-band data features from individual participants. (C) Finite element model illustrating TMS coil position on the scalp (right) and the resultant normalized electric field strength (\( E / E_{\text{max}} \)) in the cortex (left).

Discussion

We used fMRI, EEG, and TMS along with DCM to identify whole-brain networks and specific neuronal synaptic mediators within the prefrontal cortex related to age-related memory dysfunction. Whole-brain analysis from the fMRI study identified a network of cortical regions involved in the memory task that included the medial temporal, prefrontal, and posterior association cortices—3 regions typically associated with paired association tasks (Small et al. 2001; Meltzer and Constable 2005; Blumenfeld and Ranganath 2007). Within this network, we found a selective correlation with individual recall performance. Specifically, we found a strong negative correlation between the top-down connection from PFC to medial temporal cortex and memory recall whereby the stronger the connection, the worse the recall. This disruptive neurobiological “gain” is consistent with recent studies of healthy elderly individuals that have demonstrated enhanced task-dependent connectivity, compared with younger controls, between medial temporal regions and prefrontal cortex during memory encoding (Cabeza et al. 1997; Dennis et al. 2008; Oh and Jagust 2013). The finding is also consistent with observations of heightened medial temporal lobe (MTL) activity during encoding in older adults (Morcom et al. 2010; Putcha et al. 2011), which may subserve “redundancy compression” and inhibit redundant representations (Gluck and Myers 1993). In addition, we observed a significantly weaker PFC connection to VAA in the older compared with the younger cohort. Though this connection did not significantly correlate with the performance, it nonetheless suggests that the connectivity from the PFC to regions identified as active during this task is altered in the aged when compared with young cohort. Using stochastic DCM for fMRI, we extended these findings by examining the directionality of the heightened connectivity effect which showed that the PFC was the effecting source of this aberrancy. As such, we were interested in exploring the intrinsic PFC synaptic dynamics in the EEG–TMS study, where we directly manipulated intrinsic prefrontal physiology prior to the memory task using TMS and modeled the synaptic currents using DCM. Here, we found that gamma-band activity, thought the largest positive EEG correlate of fMRI BOLD responses (Rosa et al. 2010; Magri et al. 2012), had peak activations in bilateral frontal cortices. DCM for cross-spectral densities identified GABAergic connectivity to be significantly higher in the aged group compared with the young group. Given the results from the fMRI task where enhanced prefrontal connections to MTL regions were found to correlate with declines in performance, this result may seem paradoxical at first. However, it is plausible that this inhibitory age-related change is a
compensatory upregulation. Indeed, we demonstrate that cTBS removes this upregulation, but also impairs memory recall accuracy. Thus, though this ratio is not the same as in the young cohort, using TMS to adjust the ratio closer to the young is not necessarily beneficial. It has been postulated that altered cortical cellular excitation to inhibition balance may underlie some cognitive deficits (Yizhar et al. 2011). Our young cohort displayed a stable E/I ratio, whereas the aged cohorts’ E/I balance was modifiable via TMS and was different from the young cohort due to a significantly higher intrinsic inhibitory tone and an overall lower excitatory tone. These differences in PFC synaptic balance may be a reason for differences in TMS effect and crucially may lead to the altered functional modulation of both the PFC to VAA and the IFG to HPC connections when compared with the young cohort. A limitation of the study, however, is we cannot be definitive that the altered E/I ratio is a source of the network changes.

Figure 5. Excitatory and inhibitory synaptic effects within the PFC. (A) The neural mass model with specific intrinsic connectivity parameters that controlled the strength of postsynaptic responses from afferent neuronal subpopulations within a region. Gamma-band (γ) connections of interest numbered 1–5. Red connections (γ4 and γ5) denote inhibitory GABAergic connections. Black connections (γ1, γ2, and γ3) denote excitatory glutamatergic connections. (B) Group GABAergic effects within the PFC demonstrated an increased overall inhibitory tone (full red) for the aged group compared with the young group (**P < 0.05) and for differences between the aged sham and young sham groups (P < 0.05) as well as differences between the sham and TMS condition just for the aged group (**P < 0.05). (C) Group glutamatergic effects within the PFC demonstrated a main effect of age (**P = 0.026). (D) Group E/I ratios for sham (black) and TMS (gray). There was a main effect between the young and aged group (**P = 0.0417) and significant interaction whereby the TMS intervention only affected the aged group (**P < 0.05). (E) Within-subject behavioral effects from the aged cohort (n = 21), where memory performance was higher following sham TMS compared with TMS (P < 0.05, paired one-tailed t-test).
Disruption as the fMRI and EEG data were not acquired from the same cohorts. Future work will examine the sensitivity of correlations between these measures within individuals. In addition, the sex mismatch of the older cohort in the EEG study may have led to differences in effect between the young and aged groups’ response to TMS and should be considered a potential confound that could influence the between-groups differences. As concerns the TMS effect, it is currently unclear why there was only an effect in the aged cohort. This may be linked to the elevated GABAergic connectivity we found in this group or the altered E/I ratio. It is possible that an intrinsically high inhibitory state lowers the threshold for cTBS effectively serving as a prime for cTBS similar to metabolic TMS protocols. Metaplasticity is a homeostatic mechanism to ensure that one type of synaptic plasticity (potentiation or depression) does not predominate. In this case, high intrinsic inhibition combined with an inhibitory TMS protocol leads to a greater effect not a lesser one (Cassidy et al. 2014). Along the same lines, it could be that with age there are additional impaired homeostatic synaptic plasticity mechanisms. These mechanisms act to stabilize the activity of a neuron or neuronal circuit in the face of perturbation (Turrigiano 2008). Perhaps with aging, neuronal circuits have lost some function of these mechanisms, which lead to the high inhibitory connectivity and greater cTBS effect.

DCM for cross-spectral densities is designed to assay the synaptic mechanisms that generate spectral-domain data features (Friston et al. 2012). These types of models are neurobiologically grounded and are “fit” to real empirical data, so parameters can be optimized based on an individuals’ task-based response. We examined neuronal sources in the PFC with 3 distinct interacting neuronal subpopulations that together produce oscillatory responses. These models have been validated in both animals (Moran et al. 2008) and humans (Moran et al. 2014), where independent physiological measurements (microdialysis) or pharmacological perturbations verified DCM-based physiological assessments. Here, we were interested in the connectivity among the subpopulations and found an effect of age at both inhibitory and excitatory synapses, whereby inhibitory pathways were overactive and the excitatory pathways underactive in the aged cohort when compared with the younger cohort.

There are several putative mechanisms that may alter prefrontal inhibitory circuitry in the older cohort including age-related density or size of dendritic spines (Dumitriu et al. 2010) or reduced gain at inhibitory synapses due to a disruption in ascending neuromodulatory systems, for example, dopaminergic (Backman et al. 2006) or cholinergic (Dumas and Newhouse 2011) systems. These are not distinguishable from our analysis. Rather our data tie 3 levels of analysis: Behavioral performance on a memory task was related to whole-brain network circuitry, using fMRI and further, to a particular inhibitory transmission deficit upstream in the PFC using EEG-based spectral DCMs.

These results also speak to a theoretical, predictive coding account (Rao and Ballard 1999; Friston and Kiebel 2005) of aging neurobiology, which considers how top-down (prediction-related signaling) and bottom-up (error-related signaling) asymmetries may naturally emerge in an aging cortical hierarchy (Moran et al. 2014). While previously we demonstrated effective adaptations of age-related reductions in bottom-up sensory-driven plasticity, here, in the context of an associative memory task, we show that top-down signal predominance disrupts encoding with progressing age.

We present important findings related to aging neurobiology in the prefrontal cortex and its deleterious effects on memory networks. First, there is evidence of altered top-down connections from PFC to both VAA and medial temporal lobe and importantly, the altered connections to medial temporal lobe strongly associated with disrupted recall ability. Second, PFC is the source of this network disruption and this may stem from a fragile ratio of intrinsic E/I tone, where upregulated intrinsic inhibitory networks can be disrupted via external stimulation effects and could also potentially be disrupted via endogenous brain signaling. Overall, our analysis provides a holistic account of memory networks in aging given the combination of fMRI and EEG-based connectivity assessments where the former provide critical long-range connectivity measures and the latter allow for a detailed biophysical deconstruction of local cortical signals.

**Supplementary Material**

Supplementary material can be found at: [http://www.cercor.oxfordjournals.org/](http://www.cercor.oxfordjournals.org/).

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**Notes**

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**References**


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