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Corticocortical Evoked Potentials Reveal Projectors and Integrators in Human Brain Networks

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The cerebral cortex is composed of subregions whose functional specialization is largely determined by their incoming and outgoing connections with each other. In the present study, we asked which cortical regions can exert the greatest influence over other regions and the cortical network as a whole. Previous research on this question has relied on coarse anatomy (mapping large fiber pathways) or functional connectivity (mapping inter-regional statistical dependencies in ongoing activity). Here we combined direct electrical stimulation with recordings from the cortical surface to provide a novel insight into directed, inter-regional influence within the cerebral cortex of awake humans. These networks of directed interaction were reproducible across strength thresholds and across subjects. Directed network properties included (1) a decrease in the reciprocity of connections with distance; (2) major projector nodes (sources of influence) were found in peri-Rolandic cortex and posterior, basal and polar regions of the temporal lobe; and (3) major receiver nodes (receivers of influence) were found in anterolateral frontal, superior parietal, and superior temporal regions. Connectivity maps derived from electrical stimulation and from resting electrocorticography (ECoG) correlations showed similar spatial distributions for the same source node. However, higher-level network topology analysis revealed differences between electrical stimulation and ECoG that were partially related to the reciprocity of connections. Together, these findings inform our understanding of large-scale corticocortical influence as well as the interpretation of functional connectivity networks.

Key words: ECoG; effective connectivity; functional connectivity; graph theory; stimulation

Introduction

Methodological advances in functional magnetic resonance imaging (fMRI; Fox and Raichle, 2007; Biswal et al., 2010), electrocorticography (ECoG; Kramer et al., 2010; Chu et al., 2012), MEG (Bassett et al., 2006), and MRI-based tractography (Hagmann et al., 2008) have renewed interest in large-scale mapping of brain networks and their functional architecture. This has been fueled by the analysis of high-dimensional data, with graph theoretic tools a prominent example (Bullmore and Sporns, 2009). Within a graph (or network) framework, brain regions are treated as nodes and their connections as edges between nodes. Such studies of brain networks have provided new insight into the interactions that underlie cortical information processing and the pathophysiology of neuropsychiatric disease (Bassett et al., 2008; Buckner et al., 2009; Honey et al., 2009).

The direction of information flow is a facet of this research that has been difficult to ascertain. This is because connectivity measures in humans, including resting fMRI and diffusion tensor imaging to measure functional and anatomical connectivity, respectively, cannot resolve the direction of corticocortical or subcortical interactions. Anatomical tracer studies can elucidate fine-grained directional connections in experimental animals (Felleman and Van Essen, 1991) but are more difficult in humans (Burkhalter and Bernardo, 1989). A number of noninterventional methods, such as Granger causality and dynamic causal modeling, can demonstrate causal interactions by statistical inference (Oya et al., 2007; Yan and He, 2011), but may be difficult to confidently interpret (Smith et al., 2011).

Direct cortical stimulation provides an interventional method to test causal relations (or “effective connections”) between brain regions. Electrical stimulation at one location on the neocortex can trigger an electrical response at a remote location in proportion to the strength of the effective connection between the two
locations. These corticocortical evoked potentials (CCEPs) can predict resting fMRI interactions (Keller et al., 2011) and exam-
function (Matsumoto et al., 2004; Conner et al., 2011) and pathological cortical networks (Valentin et al., 2005).

Pathways connecting cortical regions consist of distinct feedforward and feedback connections within systems such as the visual cortex with an established functional hierarchy (Fel-

nerman and Van Essen, 1991). Although bidirectional anatomical connections provide the potential for communication in both directions, bidirectional communication may often be non-
symmetric. Furthermore, these communications and their direc-
tionality may be task- and state-dependent. In fact, little is known about the large-scale reciprocity of functional connections. Mapping directed connections using an interventional technique can thus provide a new insight into interpreting large-scale brain networks.

Here, we introduce a method of deriving robust effective con-
nectivity networks with high spatiotemporal resolution. By ap-
plying graph theoretic measures, we identify motor and language
systems to be highly central and project influence, whereas su-
perior parietal, superior temporal, and anterolateral frontal regions
receive influence. Finally, we report differences in the recipro-
city of effective connections that may account for distinct topologies observed between functional and effective connectivity maps. These findings provide insight into the large-scale information processing architecture of the human cortex and deepen our un-
derstanding of networks derived from measures of functional connectivity.

Materials and Methods

Subject selection. Fifteen subjects (11 female, aged 31.9 years; range 17–
60) with medically intractable epilepsy at the North Shore LIJ Compre-
hensive Epilepsy Centers participated. Patient characteristics are described in Table 1. All subjects provided informed consent as mon-
tored by the local Institutional Review Board and in accordance with the ethical standards of the Declaration of Helsinki. The decision to implant, the electrode targets, and the duration of implantation was made entirely on clinical grounds without reference to this investigation.

Electrode implantation and recording. Patients were implanted with
infracranial subdural grids, strips, and/or depth electrodes (Integra Life-
sciences) for 5–10 d. Monitoring occurred until sufficient data were col-
lected to identify the seizure focus, at which time the electrodes were removed and, if appropriate, the seizure focus was resected. Continuous infracranial video EEG monitoring was performed using standard re-
cording systems (XLTek EMU 128 LTM System, Natus Medical), sam-
ped at 2 kHz and bandpass filtered (0.1–1 kHz). A strip electrode screwed into the frontal bone near the bregma was used as common mode ground. Acquired data were notch filtered (60 Hz) and refer-
enced by subtracting the common average to remove non-neuronal ac-
tivity (Kanwisher et al., 1997). Electrodes involved in the seizure onset zone, as determined by an epileptologist blinded to the study, were re-
moved from the analysis of the majority of this study, with exception in the test for excitability (see Fig. 4).

Electrode registration. The electrode registration process has been de-
scribed previously (Keller et al., 2011). Briefly, to localize each electrode anatomically, subdural electrodes were identified on postimplanta-
tion CT with BioImagesuite (Duncan et al., 2004) and were coregistered
first with the postimplantation structural MRI and subsequently with the preimplantation MRI to account for possible brain shift caused by elec-
trode implantation and surgery (Mehta and Klein, 2010). Following coregistration, electrodes were snapped to the closest point on the recon-
structed pial surface (Dale et al., 1999) of the preimplantation MRI in
MATLAB (Dyckstra et al., 2012). Intraoperative photographs were used to corroborate this registration method based on the identification of major anatomical features. Automated cortical parcellations were used to relate electrode data to anatomical regions (Fischl et al., 2004).

Functional stimulation mapping. To localize eloquent cortex for clini-
cal purposes, electrical stimulation mapping (ESM) was performed ac-
tording to standard clinical protocol (bipolar stimulation, 2–5 s duration, 3–15 mA, 100 us/phase, 20–50 Hz). Language regions were
identified when stimulation resulted in a language deficit (expressive, receptive naming, or reading). Motor regions were identified when stim-
ulation resulted in contraction of isolated muscle groups.

CCEPs. CCEP mapping was performed with bipolar stimulation of
each pair of adjacent electrodes with single pulses of electrical current (10
mA, biphasic, 100 μs/phase, 20 trials per electrode pair) using a Grass S12
cortical stimulator (Grass Technologies). Intersetimulation interval was 1 or 2 s (5 and 10 patients, respectively). Differences in interstimulation interval had no effect on evoked potentials. The current magnitude of 10 mA was chosen, as this was the maximum current that did not induce epileptiform discharges in areas outside of the seizure onset zone. Stim-
ulation was performed extraoperatively once seizures had been recorded and antiepileptic medications had been resumed; this was typically 7–10 d after the electrode implantation surgery. Patients were awake and at rest at the time of CCEP recording.

CCEPs in human cortex generally consist of an early sharp response (10–50 ms poststimulation) and a later slow-wave (50–250 ms). These responses have previously been referred to as N1 and N2, respectively, due to the existence of negative voltage deflections during these time periods (Matsumoto et al., 2004). However, as the deflections observed during these time periods are highly variable in both polarity and latency, and as negative deflections are often followed by positive deflections and vice versa, we chose to examine the magnitude of the response of evoked potentials regardless of polarity. Therefore, we refer to the early and late responses as A1 and A2 (A for absolute magnitude). In support of this change, a previous study demonstrates a similar spatial correlation be-
tween CCEP and resting fMRI when using the N1 or P1 response (Keller et al., 2011).

For each stimulation and response site, mean evoked potentials (from 20 repetitions) were converted to a Z-score based on the peak amplitude re-

dorse relative to the prestimulus baseline (−500 to −5 ms) for the early
(A1) and the late (A2) response. The first 10 ms following stimulation was
excluded from analysis because of stimulation artifact. Evoked potentials that did not switch polarity following the stimulation artifact were also re-
jected as these were most often seen when the amplifier failed to return from saturation. Responses within 1.5 cm of the stimulation site were removed to reduce the contribution of volume conduction. We would like to emphasize that the Z-score is calculated based on the evoked potential at each site and is independent of responses at other sites.

To determine the threshold for significant evoked responses, receiver operating characteristics are highly variable in both polarity and latency, and as negative deflections are often followed by positive deflections and vice versa, we chose to examine the magnitude of the response of evoked potentials regardless of polarity. Therefore, we refer to the early and late responses as A1 and A2 (A for absolute magnitude). In support of this change, a previous study demonstrates a similar spatial correlation be-
tween CCEP and resting fMRI when using the N1 or P1 response (Keller et al., 2011). A Z-score of 6 was de-
graph theoretic measures were used to characterize network topology. To apply these graph theoretic measures, it implies that our parameters provided a spatial response map that is representative of the connection between node i and node j. To ensure that stimulated and recorded responses were spatially consistent. The weighted, asymmetric matrix of CCEPs was then converted to a binary, asymmetric matrix by thresholding based on the ROC analysis described above and further characterized using a variety of network measures implemented in MATLAB (MathWorks) in the Brain Connectivity Toolbox (Rubinov and Sporns, 2010). Graph theory measures used to characterize each region in the network included: outdegree, the total number of significant CCEPs observed when the region of interest is stimulated; indegree, the total number of times stimulation of any region evokes a significant CCEP at the region of interest; degree centrality, the number of total suprathreshold evoked responses (indegree + outdegree); flow, the difference between the amount of outgoing and incoming connections (outdegree − indegree); reciprocity index (B), the proportion of time a recurrent suprathreshold CCEP is present when one suprathreshold CCEP is observed in either direction; path length, the number of shortest connections (suprathreshold CCEPs) needed to travel from one region to another (a measure of long-range connectivity); and clustering coefficient, the proportion of a region’s neighbors which exhibit suprathreshold CCEPs (a measure of short-range connectivity). As the total number of connections is identical for each stimulation site, calculation of the total number or percentage of connections will yield equivalent results. Whole brain networks are described by density (k), the number of connections in the matrix divided by the total number of possible connections; and small worldiness, the extent to which a network has a higher clustering coefficient and shorter path length when compared with a random network with an equal number of overall connections. Networks with high clustering coefficient are thought to exhibit high local efficiency information processing, while those with a short path length represent efficient global processing as it takes fewer steps to travel from one node to the next. Measures derived from CCEPs will be referred to as “causal” (causal degree, causal indegree, causal outdegree, causal flow) as each edge in the matrix represents the directional influence of one node on another (Seth et al., 2005).

Modeling reciprocity. Identifying reciprocated and nonreciprocated influence can help in identifying the channels of information flow across the network. However, some amount of reciprocity is expected, even in a randomly connected network, and this baseline level of reciprocity rate depends on the network density. Therefore, to determine whether the proportion of reciprocal effective connections differ from chance, we constructed a model based on the empirical network density. We first categorized stimulus-response electrode pairs according to their Euclidean distance (“short-range” pairs <5.0 cm; “long-range” pairs >5.0 cm). If p represents the total number of stimulation–response pairs which exhibits at least one suprathreshold CCEP connection, and q represents the total number of pairs made up of reciprocated bidirectional connections, then the probability that a random connection is reciprocated (i.e., part of a bidirectional pair) is as follows:

\[ B = \text{reciprocity index} = q/p. \]
Hilbert transformed to obtain the envelope of the signal (high gamma bandpass Butterworth filter). It is important to emphasize that HGP is used here as a proxy to analyze low-frequency fluctuations of the gamma bandpass Butterworth filter. For each of 1000 simulations, a network was populated such that the total number of directed edges was randomly assigned between the total number of nodes (i.e., stimulating–recording pairs). For each stimulation site, the number of significant connections for the simulation was made equal to that of the experimental data. Then, the reciprocity index for the model was calculated and results were compared with experimental CCEP networks for each subject. This analysis was performed separately for short-range and long-range connections. Additionally, to determine the effect CCEP threshold has on reciprocity, this analysis was repeated for less and more stringent significance thresholds ($z = 2, 4, 6, 8, 10, 12, 14$).

Reciprocity: regional analysis. Because performing reciprocity analysis on individual electrodes is likely to underestimate long-range connectivity, we also used a regional-based approach. Each electrode was assigned to nearest anatomical parcellation as described earlier (see Electrode registration; Fischl et al., 2004). A significant connection between two anatomical regions was defined in the following manner. Significance between CCEP stimulation–responses between anatomical regions was averaged to create the mean CCEP regional response. A distribution of all mean CCEP regional responses were computed, and a group CCEP regional response threshold was defined as $>2$ SD from this distribution. In this manner, the threshold for significance of the mean CCEP regional response was calculated to be $z = 5.42$. This threshold was used to create binary regional-based connectivity matrices. Finally, the mean reciprocity for short-range ($<5$ cm) and long-range ($>5$ cm) anatomical regions was calculated. For the parcellation-based analyses, the same distance criterion was used such that if the Euclidean distance between the center of parcellations was calculated to be $>5$ cm, it was defined as a long-range connection. A control analysis similar to that used in the electrode-based analysis was computed. The resultant electrode-based null connectivity matrix was then grouped by regions in a similar manner as described above for the experimental data.

Resting ECoG. The resting ECoG protocol was described previously (Keller et al., 2013). ECoG was acquired for 3–6 min while subjects were asked to rest quietly. Intertial discharge-free periods (276.1 ± 71.2 s SD) were selected for analysis. Recording sessions were conducted $>2$ h before or after an ictal event to avoid preictal or postictal changes that may alter cortical connectivity, and before electrical stimulation mapping. Channels with high amplitude noise (SD $>250$ uV) as well as electrode sites corresponding to the seizure onset zone were excluded (mean 5.6 ± 3.2% of all channels). The remaining channels were notch-filtered to remove power line noise and referenced by subtracting the common average reference. Pre-stim random (50 ms; FWHM 50 mm; Miller et al., 2007; Dykstra et al., 2012). Slow fluctuations (0.1–1 Hz) were filtered to leave only the strongest 5% of connections. The correspondence between modalities of single-site connectivity profiles was assessed by calculating the spatial correlation between the connectivities of each ROI (seed electrode for ECoG calculations; stimulation electrode for CCEPs) and all other electrodes (ECoG correlation with seed electrode, evoked response for CCEPs). These correlation values were then averaged across all ROIs and across subjects. To compare the global connectivity profiles across modalities, we used a group surface-based analysis. For each patient, the $z$-normalized network measure was plotted after convolution with a 3D Gaussian smoothing kernel (FWHM 50 mm; Miller et al., 2007; Dykstra et al., 2012). Smoothed network measure maps for each subject were then transformed to the group-averaged cortical surface. Group surface maps of ECoG degree and CCEP degree, indegree, outdegree, and net flow were then compared. As a further analysis, for each cortical parcellation, graph theory measures at electrodes found within a region were averaged together and the correlation coefficient was calculated for each parcellation-based ECoG and CCEP network measure. To determine whether these ECoG findings were specific for high-gamma, we repeated this analysis of the ECoG looking at the correlation coefficient between the raw, unfiltered voltages at sites of interest. Qualitatively similar results were obtained with respect to correspondence of ECoG and CCEP (see Fig. 8A, B).

Results

We examined directed networks derived from electrically evoked potentials recorded from subdural electrodes in 15 subjects undergoing intracranial monitoring for surgical evaluation of epilepsy (clinical information and demographics are presented in Table 1). In total, 1384 cortical sites were probed. The workflow for this analysis is depicted in Figure 1 (see Materials and Methods). Briefly, single-pulse stimulation elicited evoked potentials (CCEPs) that were converted to Z-scores based on the response amplitude of the early (<50 ms, A1) segment of the CCEP. Each stimulation and associated responses represent one row in the connectivity matrix. This weighted connectivity matrix was thresholded and graph theoretical measures were calculated to quantify network topology, directionality, and reciprocity.

Directed networks derived from CCEPs are reproducible and exhibit small world topology

Following the construction of CCEP directed networks, we examined network topology and determined its sensitivity to distance and network density. Degree was calculated as a function of distance from the stimulation site and response amplitude. CCEP networks were composed of abundant short-range connections and few long-range connections (Fig. 2B). The relationship between network density ($k$) and significance threshold of the evoked potential ($z = 0.5-14$) is depicted in Figure 2C. At high response thresholds, the density of the network decreases as ex-
expected. We next determined whether CCEP networks exhibit small-world topology, a feature of human brain networks characterized by a higher-clustering coefficient and shorter path length compared with random networks (Sporns and Honey, 2006; Achard and Bullmore, 2007; Bassett et al., 2008). For each response threshold, clustering coefficient and path length were normalized to 100 random networks with equivalent total indegree and outdegree as the CCEP network. CCEP directed networks exhibited small world properties in the range of $z = 6 – 14$, with a mean clustering coefficient of 2.0 in this range of thresholds (Fig. 2C). This measure is in general agreement with reports from other human brain networks (Achard and Bullmore, 2007; Bassett et al., 2008; Yan and He, 2011).

We next examined how network properties varied as a function of the threshold used to binarize the CCEP network. At each node, outdegree measures at a threshold of $z = 6$ were plotted against those at $z = 10$ (Fig. 3D). A linear relationship ($r = 0.96$, $p < 0.01$) was observed, supporting the notion that CCEP networks are largely density insensitive in the threshold range that exhibit small world topology. Then, we investigated the extent to which network properties derived from CCEPs are influenced by distance between the stimulating and recording electrode. One would predict that electrodes close to the stimulation site would exhibit larger evoked potentials, and thus electrodes with the most neighbors (i.e., at the center of the grid) exhibit the highest number of significant connections. To estimate the effects of this distance bias, we generated simulated data from a model in which CCEP amplitude was inversely proportional to the distance from the stimulation site (Fig. 3E). As expected, the nodes with highest degree under this model were located in the center of the grid. The distance effect does not resemble the empirical data, however, and in particular it cannot explain how regions with the highest degree empirically were located at the corner of the grid, with few proximal electrodes (Fig. 3E). Thus, distance alone does not account for the network topographies we report.

Network analysis of CCEPs reveals projectors and integrators of neocortical circuits

To examine the topological organization of the cortex, we calculated network measures including causal indegree, outdegree, degree, and net flow at each electrode. Analysis from a single subject demonstrates the transformation from the suprathreshold CCEP response profile at a single stimulation site (Fig. 5A, B) to the cortical representation of network measures (Fig. 5C). In this subject, regions of high outdegree and degree centrality are localized to sensorimotor regions, whereas temporal lobe nodes exhibit high indegree. Causal flow (outdegree – indegree) was in this subject, outward at sensorimotor cortex and inwards in the temporal lobe (Fig. 5C). Examples from six subjects illustrate consistently high outdegree measures in para-central cortex (Fig. 6). Indegree, which exhibited a less consistent topography across subjects, is discussed below.

To provide a subject-averaged measure of the key projectors and integrator nodes, network measures were averaged across cortical regions. After determining the cortical area where each electrode was implanted based on the cortical parcellation procedure (see Materials and Methods), we calculated the mean network measures across all electrodes in each region (Fig. 7). The precentral gyrus, the postcentral gyrus, lingual gyrus, and the temporal pole exhibited the highest causal outdegree, and casual outflow. The rostral and caudal middle frontal gyrus and superior and inferior regions of parietal cortex exhibited the highest indegree, and also exhibited net inflow of influence.

Relationship to excitability

It is important to minimize the possibility that the reported regional differences in causal influence are not driven by differences in the excitability of the neural tissues beneath the stimulated elec-
trodes. We aimed to rule out this possibility in two ways. First, we examined the relationship between network measures and local excitability, where local excitability was estimated using the CCEP profile of nearby electrodes. Second, we examined the indegree and outdegree of seizure onset zones, which are known to exhibit increased excitability (Valentín et al., 2005; Enatsu et al., 2012).

First, to investigate excitability via local CCEP connectivity, we computed the (1) mean magnitude of local (within 15 mm) CCEP responses upon stimulation of each site and (2) the network measure (degree, indegree, outdegree) associated with that site. Within all individual subjects, and for both the A1 and A2 poststimulation interval, no significant relationship existed between the mean local connectivity around each stimulation site and the node degree \( r_{\text{A1 degree, local}} = 0.03; r_{\text{A2 degree, local}} = 0.04 \), outdegree \( r_{\text{A1 outdegree, local}} = 0.06; r_{\text{A2 outdegree, local}} = 0.07 \), and indegree \( r_{\text{A1 indegree, local}} = 0.04; r_{\text{A2 indegree, local}} = 0.02 \).

Second, to relate intrinsically excitable tissue within the seizure onset zone to network measures, we characterized the CCEP indegree and outdegree within the seizure onset zones identified within each individual subject. Seizure onset zone regions exhibited higher indegree than regions not involved in the seizure onset \( p < 0.05 \). No significant differences were observed between seizure onset zone regions and outdegree. Similar results were observed when using the non-\( z \)-transformed and the \( z \)-transformed CCEP amplitudes (results reported were based on non-\( z \)-transformed amplitudes).

Relating network measures to functional traits of cortical subsystems

We next asked whether underlying cortical function and anatomy relates to the strength of evoked potentials in that region. We defined a region to be involved in a certain function if high-frequency ESM of that region elicited a behavioral response (e.g., speech arrest, hand motor response). Electrodes that elicited motor responses during ESM exhibited significantly higher causal indegree, outdegree, degree centrality, and net outflow compared with electrodes not involved in movement \( p < 0.01 \), two-tailed \( t \) test). Electrodes involved in language (expressive or receptive speech) exhibited significantly higher causal indegree, degree centrality, and causal outflow compared with electrodes not involved in these functions (Fig. 7).

Figure 4. Relationship of seizure onset zone to CCEP networks measures. Relationship between the degree, indegree, and outdegree at electrodes in the seizure onset zone compared with those outside the seizure onset zone. Error bars represent SEM, \( * p < 0.05 \).

Distinct functional and effective connectivity profiles

How do directed, effective connectivity measures compare to undirected, functional connectivity measures? To investigate this, we compared the single-site and global connectivity profiles...
of CCEPs (effective connectivity) and ECoG (functional connectivity). The single-site connectivity profiles are the set of connections between a given electrode pair and all others, while the global connectivity profiles reflect overall connectedness regardless of spatial distribution (see Materials and Methods). Across all ROIs and patients, the mean correspondence between local connectivity profiles (or the spatial correspondence of network measures) for ECoG and CCEP networks were $r = 0.38$ (range $r = 0.25–0.52$ across patients) for the A1 timeframe and $r = 0.36$ (range $r = 0.23–0.54$) for the A2 timeframe. However, because these correlations are computed on local connectivity profiles, they normalize the mean connectivity of each node, and do not indicate whether global network features (such as degree) are shared across the ECoG and CCEP networks.

Therefore, we next investigated the relationship between global connectivity profiles of CCEP and ECoG networks by creating group-based surface maps (see Materials and Methods). ECoG network analysis revealed high degree centrality in the anterior temporal, prefrontal, and superior parietal regions (Fig. 8A). CCEP network analysis revealed regions of strong causal

Figure 5. Graph theory measures in one subject. Examples of nodes with high causal (A) outdegree and (B) indegree. For each brain, only suprathreshold CCEPs are plotted and represented with a line connecting the stimulating electrode to the response site. C, Network measures across all stimulation sites. Network measures are represented at each node with a heat map according to its $z$-thresholded network measure.

Figure 6. Causal outdegree measures across subjects. Note the strong outdegree around the central sulcus. Warm colors represent regions with strong outdegree.
degree, outdegree, and outflow localized to precentral and postcentral gyrus, supplementary motor area, and posterior MTG and ITG (Fig. 8C). High causal indegree was observed in precentral and postcentral, parietal, and prefrontal regions, and causal inflow was localized to prefrontal, parietal, and anterior STG (Fig. 6B). Compared with resting ECoG degree, CCEP outdegree, degree, and flow exhibited a strong negative correlation (Fig. 8D; $r_{\text{ccep outdegree, ecog degree}} = -0.60$; $r_{\text{ccep degree, ecog degree}} = -0.57$; $r_{\text{ccep flow, ecog flow}} = -0.52$).
CCEP networks exhibit low functional reciprocity between cortical regions

What could explain the differences in global connectivity between effective and functional networks? We hypothesized (resting) functional connections seen with EC0G were most likely to correspond to (stimulation-driven) effective connections seen with CCEP when the effective connections were reciprocal. To test this hypothesis, we first had to characterize the proportion of bidirectional interactions (reciprocity index, $B$) across nodes and subjects in our CCEP data.

In each subject, the level of reciprocity varied widely across nodes (Fig. 9A, B). Across all subjects and nodes, the mean reciprocity ($B_{\text{mean}}$) was 9.4% (range, $B = 0.0$–50.2%, 11.1% SD). In both the empirical CCEP data as well as the simulated CCEP data examining reciprocal interactions, reciprocity decreased as distance from the stimulation site increased (Fig. 9C, D). Short-range (<5 cm) reciprocity was found to be significantly higher than predicted by the control analysis (see Materials and Methods). Across all subjects in the A1 timeframe (Fig. 9C; CCEP$_{\text{mean}} = 24.1\%$, model$_{\text{mean}} = 10.8\%$, $p < 0.001$, two-tailed $t$ test), whereas long-range connections did not exhibit a significant change in reciprocity compared with the control analysis (Fig. 9D; CCEP$_{\text{mean}} = 9.1\%$, model$_{\text{mean}} = 9.7\%$). The A2 timeframe demonstrated similar findings (data not shown). To ensure that the cutoff for defining significance did not affect these results, we recalculated reciprocity using three levels of threshold. As expected, reciprocity increased for lower thresholds CCEP$_{1} = 6$ = 24.1% (±5.2 SE); CCEP$_{2} = 4$ = 29.5% (±4.6 SE); CCEP$_{3} = 2$ = 46.2% (±4.8 SE) for short-range connections and CCEP$_{1} = 4$ = 9.1% (±3.1 SE); CCEP$_{2} = 4$ = 12.1% (±4.9 SE); CCEP$_{3} = 4$ = 29.7% (±6.1 SE) for long-range connections. For each threshold, short-range reciprocity was significantly higher than expected from a random network model, while long-range reciprocity was not. For the regional-based reciprocity analysis, reciprocity was higher than for the electrode-based approach, with CCEP regional-based reciprocity at 73.1% and 42.3% for short- and long-range connections, respectively (Fig. 9C, D). Both short- and long-range connectivity did not exhibit higher reciprocity than expected given the degree distribution of the network.

Reciprocity of stimulation-evoked responses predicts the strength of spontaneous interareal correlations

Having mapped the reciprocity of CCEP effective connections, we investigated its relationship with interareal resting EC0G functional connectivity. One might hypothesize that specific reciprocal connections are sites of important functional interaction, which may be reflected in stronger functional connectivity (i.e., dynamical correlation) between regions. Figure 10A illustrates CCEP input maps (examining the evoked response measured at the center node when stimulating other regions) as well as CCEP output maps (examining the CCEP response measured when stimulating the center node) and EC0G functional connectivity maps for a range of electrodes. Across subjects, regions of strong EC0G correlation demonstrated larger CCEP responses than those regions of weak EC0G correlation (Fig. 10B, top; $p < 0.001$). Note in Figure 8A longer-range stimulation-evoked responses (unidirectional connections) occasionally corresponded to a strong EC0G correlation (black arrowhead), but edges with reciprocal CCEP connectivity (overlap between CCEP inputs and outputs) were more likely to exhibit EC0G correlation (white arrowheads). To quantify this, we mapped the strength of the resting EC0G connectivity as a function of the type of CCEP connection (bidirectional significant connection, unidirectional significant connection, no significant connection). Across subjects, for short distances (<5 cm), bidirectional CCEPs mapped to regions of the strongest EC0G correlations, followed by unidirectional connections.
directional responses, with regions of subthreshold CCEP responses corresponding to the weakest ECoG correlation values (Fig. 10C, top; \( p < 0.01 \) corrected for after multiple comparisons). This trend was consistent in 12/15 subjects before normalization (\( p = 0.02 \), paired \( t \) test). In contrast, for long-range connections (> 5 cm), the strength of ECoG correlations did not vary as a function of the reciprocity or the presence of CCEP connections (Fig. 10B, C; bottom). For the regional-based analysis, long-range bidirectional CCEPs between parcellations exhibited stronger resting ECoG connectivity compared with unidirectional and no significant CCEPs. However, no significant difference was observed for short-range connections (Fig. 10D).

Discussion

This study provides important insights into the directedness of networks in human cortex using direct stimulation and recording. Our findings can be summarized as follows: (1) peri-Rolandic cortex and frontal and temporal regions that were identified to have language or motor function with electrical stimulation mapping, exhibited the highest causal outdegree, centrality, and projected influence whereas the superior parietal, lateral temporal, and lateral prefrontal regions exhibited strong causal indegree and received influence; (2) maps of effective and functional connectivity demonstrated positively correlated single-site connectivity profiles but negatively correlated overall topology; and (3) functional corticocortical reciprocity across all regions was low, decreased with distance, and at short distances reciprocal connections were associated with strong interareal interactions at rest.

Language and sensorimotor networks: central cortical hubs?

It has long been known that the motor cortex projects a copy of internally generated movement to other sensory systems to estimate the intrinsic response and measure the influence from external stimuli. Although the behavioral effect of this “corollary discharge” or “effference copy” is well described, the neural representation of these projections are not well characterized in humans (Poulet and Hedwig, 2007). This would likely manifest in outgoing projections from motor cortex to a diverse array of cortical and subcortical regions. We observed sensorimotor regions exhibiting abundant connections to other cortical regions in language, somatosensory, auditory, and visual cortex. It is possible this observation may represent the neural correlate of the corollary discharge; however, further work coupling electrophysiology with behavioral studies is necessary to experimentally validate this finding. Nevertheless, the high centrality of motor cortex demonstrates that internal motor representation appears to be a ubiquitous feature of functional networks.

It is not likely that differences in the topology of networks across cortical regions can be attributed to the intrinsic excitability of the stimulated region. First, no relationship between the strength of neighboring CCEPs and outdegree measures was observed, supporting the notion that changes in excitability do not underlie differences in network measures. Second, seizures arise from the imbalance of excitation and inhibition that can result in high intrinsic excitability within the seizure onset zone (Valentin et al., 2005). Therefore, if CCEPs reflect the intrinsic excitability of a given region, stimulation of the seizure onset zone should result in stronger and more abundant CCEPs at other regions. To the contrary, we found slightly higher indegree but no difference in outdegree in the seizure onset zone. Together, these findings suggest that regions of high outdegree including sensorimotor and posterior temporal regions does not reflect differences in excitability and instead are likely the major cortical projectors of the brain.

Asymmetry in large-scale networks

Although it is well established that the majority of synaptic connections in the brain are reciprocal in nature (Felleman and Van Essen, 1991), an asymmetric global connectivity would allow the
efficient processing, integration, and storage of incoming sensory stimuli. We observed a high degree of asymmetry (low reciprocity) among large-scale cortical circuits. As expected, this level of reciprocity was higher at lower significance thresholds. Tracer studies largely focus on local connectivity within a given sensory region, which tends to be strongly interconnected with feedforward and feedback connections (Felleman and Van Essen, 1991).

It is important to note that the presence of a reciprocal anatomical connection does not necessarily indicate a reciprocal functional or effective connection. Previous examination of the directionality of the anterior and posterior language cortices revealed that although stimulation of either language region resulted in CCEPs at the other language region, an asymmetry was observed, wherein stimulation of anterior language regions elicited stronger CCEPs in posterior language regions than anterior CCEPs elicited from stimulation of posterior regions (Matsuzumo et al., 2004). Quantification of CCEP reciprocity in a single sensory system has reported reciprocal connections 75–95% of time within the sensorimotor network, but decreased to 25–50% when evaluating reciprocity at specific electrodes (Matsuzumo et al., 2007). These reciprocity values are in line with those in the current study, especially considering that reciprocity was calculated across multiple functional systems. As previous tracer and CCEP studies examined shorter-range connectivity often focusing on a single sensory system, it is not surprising that we observed this higher level of functional asymmetry across networks.

Compared with our model, the frequency of short-range suprathreshold CCEPs was found to be higher than expected for both timeframes of the CCEP but no different for long-range interactions. The short-range observations are in line with these networks exhibiting small-world topology (Bassett and Bullmore, 2006; Sporns and Honey, 2006). It is important to note that this asymmetry between regions may not only reflect differences in direct synaptic pathways. For example, stimulation of site A may elicit evoked responses at site B through direct corticocortical pathways, whereas stimulation of site B may elicit an evoked response at site A through a cortical or subcortical intermediate region. It is also important to note that some of the reciprocal connections in the brain may be missed due to the electrode grid spacing for each patient. Nevertheless, under either interpretation, the present results constitute causal evidence of large-scale asymmetric propagation across the brain.

Reciprocity influences interareal functional connectivity

Although corticocortical interactions are largely asymmetric, their reciprocal nature appears to be associated with the strength of spontaneous interareal interactions. Network topology from effective connectivity networks (high centrality at peri-Rolandic cortex in CCEP) differed from observations in functional connectivity networks (low centrality in sensorimotor cortex and high centrality in parietal, anterior temporal, and prefrontal regions in resting ECoG) in the same subjects. The topology of functional connectivity derived from resting ECoG networks support previous literature on resting fMRI and diffusion tensor imaging which report low centrality in primary sensory regions and high centrality in the default mode network (Hagmann et al., 2008; Buckner et al., 2009; Zuo et al., 2012). The corroration of functional connectivity maps in these subjects with the literature reinforces the notion that electrode sampling bias does not confound our results. However, within-subject differences between effective and functional connectivity were unexpected. Quantification of the local and global connectivity profile of both modalities demonstrated positive correspondence between the local connectivity profiles and negative correspondence between the global connectivity profiles.

We believe that these techniques measuring slightly different neuronal processes account for the discrepancy between local and global connectivity profiles and may shed light on the neural substrates underlying resting state functional connectivity. It is first important to note that although findings presented here as well as previous studies both demonstrated a positive correspondence between CCEPs and resting state measures in local connectivity profiles (Keller et al., 2011), the strength and spatial spread of CCEPs explained only ~20% of the functional connectivity profile, suggesting that CCEPs and functional connectivity represent slightly different neuronal processes. The motor network can serve as an example to explain these discrepancies between modalities. Stimulation of the motor cortex results in strong evoked potentials both in regions exhibiting strong resting ECoG correlations but also at more distant sites exhibiting low resting ECoG correlations. These connectivity profiles result in a positive (but not very high) correspondence between modalities. In this example, a high outdegree in motor cortex for CCEPs and a low resting ECoG degree would result in a negative correspondence in global connectivity profiles. In this fashion, we believe that CCEPs may probe the complete set of available anatomical connections, whereas resting functional connectivity highlights the subset used during specific brain states. Evaluating the relationship between CCEP and resting ECoG topology during different brain states would directly test this hypothesis. Another explanation for the discrepancy between local and global connectivity profiles is that the calculation of the local connectivity profile does not account for differences in CCEP reciprocity and instead only evaluates unidirectional responses. We demonstrated that regions underlyin reciprocal effective connections exhibit stronger functional connections, suggesting that reciprocal connectivity in the brain may underlie the strength of functional interactions.

Implications and limitations

CCEP networks described here provide extensive coverage of the lateral and inferior human cortex. Although this method cannot provide whole brain coverage compared with fMRI or diffusion tensor imaging, it does exhibit three notable advantages: (1) the ability to resolve direction of flow, (2) the direct recording of neural activity on the cortical surface, and (3) high spatiotemporal resolution. Although each subject did not provide whole brain coverage, group analysis allowed the sampling of the majority of cortical regions on the lateral, medial, and inferior cortex.

Although these subjects provide access to a direct measure of neural activity in awake humans, it is difficult to interpolate findings about brain networks from these patients to the general population. However, the heterogeneous etiology and localization of seizures in the patient population, removal of electrodes in the seizure onset zone for this analysis, and the consistency of findings across subjects support the notion that these results may be interpolated with some level of confidence. Future studies will help elucidate the neural mechanism underlying CCEPs. Additionally, experiments enhancing our understanding of how behavioral states modulate functional and effective connectivity will aid in the interpretation of findings presented here.

References


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