Direct Comparison of Spontaneous Functional Connectivity and Effective Connectivity Measured by Intracortical Microstimulation: An fMRI Study in Macaque Monkeys

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Correlated spontaneous activity in the resting brain is increasingly recognized as a useful index for inferring underlying functional-anatomic architecture. However, despite efforts for comparison with anatomical connectivity, neuronal origin of intrinsic functional connectivity (inFC) remains unclear. Conceptually, the source of inFC could be decomposed into causal components that reflect the efficacy of synaptic interactions and other components mediated by collective network dynamics (e.g., synchronization). To dissociate these components, it is useful to introduce another connectivity measure such as effective connectivity, which is a quantitative measure of causal interactions. Here, we present a direct comparison of inFC against emEC (effective connectivity probed with electrical microstimulation [EM]) in the somatosensory system of macaque monkeys. Simultaneous EM and functional magnetic resonance imaging revealed strong emEC in several brain regions in a manner consistent with the anatomy of somatosensory system. Direct comparison of inFC and emEC revealed colocalization and overall positive correlation within the stimulated hemisphere. Interestingly, we found characteristic differences between inFC and emEC in their interhemispheric patterns. Our results suggest that intrahemispheric inFC reflects the efficacy of causal interactions, whereas interhemispheric inFC may arise from interactions akin to network-level synchronization that is not captured by emEC.

Keywords: causal connectivity, functional magnetic resonance imaging, somatosensory

Introduction

Functionally related brain regions often show correlations in spontaneous blood oxygen level-dependent (BOLD) activity as measured by functional magnetic resonance imaging (fMRI) (Biswal et al. 1995). This intrinsic functional connectivity (inFC) has been increasingly used in research and becoming an useful tool for noninvasively assessing functional network organization in the intact human brain (Fox and Raichle 2007; Van Dijk et al. 2010). Using inFC, previous studies have revealed discrete groups of brain regions forming "resting-state networks" (Greicius et al. 2003; Fox et al. 2006; Smith et al. 2009). Similar functional networks were found to exist across different arousal states (Fukunaga et al. 2006; Greicius et al. 2008) and in different species (Vincent et al. 2007; Pawela et al. 2008). More recently, pathological changes in inFC have been reported in patients with Alzheimer’s disease (Buckner et al. 2009) and cognitive impairment such as Attention Deficit/Hyperactivity Disorder (Wang et al. 2009), suggesting the potential clinical importance of inFC (Bassett and Bullmore 2009; Zhang and Raichle 2010).

However, despite its prevalence and wide application, the origin and biological significance of inFC remain relatively unclear (Biswal et al. 2010). Although several studies have shown a close correspondence between inFC and anatomical connections (Koch et al. 2002; Hagmann et al. 2008), recent studies reported that the presence of strong inFC between 2 regions does not always coincide with the presence of anatomical connections between them (Vincent et al. 2007; Shmuel and Leopold 2008; Honey et al. 2009). These and other modeling studies (Honey et al. 2007; Deco et al. 2009) suggest that inFC captures network-level synchronization that is loosely constrained by anatomical connectivity. However, since inFC is, by definition, a mere temporal correlation between 2 neurophysiological signals without the requirement for causal relationship between them (Friston et al. 1993; Lee et al. 2002), it is difficult to specify the source that gives rise to inFC. For example, it is difficult to distinguish between the component of inFC arising from causal interactions mediated through synaptic connections and the other component mediated by complex network-level synchronization emerging from weakly connected neuronal networks (Honey et al. 2007). Important information missing here is the efficacy of synaptic connections that could convey causal influence of a neuronal population exerted over another.

Effective connectivity is a potential alternative connectivity measure, providing a quantitative measure of causal interactions between neuronal populations (Friston et al. 1993; Lee et al. 2002). By using direct perturbation of local neuronal activity by electrical microstimulation (EM), effective connectivity can be measured in an entirely model-free paradigm (Massimini et al. 2005; Driver et al. 2009). Moreover, by combining EM and fMRI, it is possible to map EM-derived effective connectivity (emEC) in the whole brain using the same physiological measure (i.e., the BOLD signal) that is used in inFC (Tolias et al. 2005; Ekstrom et al. 2008; Fiedl et al. 2008; Moeller et al. 2008; Driver et al. 2009). These features make emEC the ideal candidate for direct comparison with inFC.

In the present study, we conducted a direct comparison of inFC and emEC in the somatosensory system of macaque monkeys. We first conducted whole-brain mappings of emEC using simultaneous EM and fMRI. We chose the primary somatosensory cortex (S1) as the target of EM for its well-known anatomical profile (Burton and Fabri 1995; Burton et al. 1995). Subsequently, we conducted functional connectivity (fc)-fMRI experiments using the same monkeys and mapped inFC between the stimulated part of S1 and the whole brain. Use of the same individual animals and experimental setups allowed us to make a direct comparison of the 2 types of...
connectivity. Moreover, the common physiological measure (BOLD) used to estimate inFC and emEC enabled us to compare the 2 measures on voxel-by-voxel basis. Our main interests were 2-fold: First, we investigated overlaps and correlations in inFC and emEC; Second, we investigated regions where inFC and emEC did not overlap and tried to specify a feature that significantly differentiated inFC from emEC.

**Materials and Methods**

**Animals**

Two male macaque monkeys were used in this study (M1 and M2; *Macaca mulatta*; weighing 5.5 and 6.5 kg, respectively). Monkeys underwent surgical procedure prior to the experiments and were implanted MRI-compatible custom made head-holding devices and recording chambers (Christ Instruments). Both devices were attached to the skull under aseptic conditions, using ceramic screws (Japan Medical Materials), custom made attachments made of polyether ether ketone (Sanwa Techno) that bridged and connected the devices to the skull, and a small amount of dental acrylics. In the surgery, general anesthesia was maintained with pentobarbital (4 mg/kg/h, intravenous injection) and xylazine (2 mg/kg, intramuscular injection), supplemented as needed. During the postsurgical recovery period, monkeys received analgesics (acetaminophen, 20 mg/kg/day or pranoprofen, 3 mg/kg/day) for at least 3 days and prophylactic antibiotics (ampicillin, 100 mg/kg/day, intramuscular injection or enrofloxacin, 5 mg/kg/day, subcutaneous injection) for 1 week. All experimental protocols were in full compliance with the regulations of The University of Tokyo School of Medicine and with the NIH guidelines for the care and use of laboratory animals.

**4.7-T fMRI Acquisition**

Monkeys were scanned with a 4.7-T MRI scanner (BioSpec 47/40, Bruker BioSpin) with an actively shielded gradient coil (100 mT/m) and a quadrature surface RF coil (QDSC) or a U-shaped volume coil. For functional scans, the QDSC and a single-shot gradient echo-planer imaging (EPI) sequence were used (repetition time: 2.5 s; echo time, 21 ms; flip angle, 80°; matrix size, 96 x 64; voxel size, 1.5 x 1.25 x 1.5 mm³; 25 axial slices). Anatomical images for each experimental session were obtained using a T₁-weighted fast spin echo sequence (0.75 x 0.625 x 1.5 mm³). High-resolution anatomic images for template images were obtained using the U-shaped coil and a T₁-weighted gradient echo sequence (0.5 mm isotropic).

**Electrical Microstimulation-Functional Magnetic Resonance Imaging**

Shortly after the postsurgical recovery period and a few days prior to the simultaneous EM and fMRI experiments (EM-fMRI), small craniotomies (~3 mm) were made over the postcentral gyrus under aseptic conditions. At the beginning of each EM-fMRI session, the monkey was anesthetized with an intramuscular injection of medetomidine/midazolam (30 μg/kg and 0.5 mg/kg, respectively) and transferred to a shielded room for electrophysiology outside the MRI scanner. Prior to electrode insertion, the exposed skull in the recording chamber was covered by 2% agarose. Agarose in the chamber also reduced susceptibility artifacts due to the presence of the recording chamber. Thus, inside the MRI scanner, the monkey was placed in a supine position with its head position stabilized using the head-holding devices. Since the electrode was inserted to the brain in the dorsoventral direction, both the stimulation and the reference electrodes became parallel to B₀ and the distortion of the magnetic field due to these electrodes was minimized. After this minimization procedure, susceptibility artifacts due to the presence of the microelectrode became very small (Supplementary Fig. 2) with respect to the spatial resolution of the EPI images (1.5 x 1.25 x 1.5 mm³) and to the size of spherical seed region of interest (ROD) (4 mm in diameter; see below for details) used for the calculation inFC.

During fMRI scanning, anesthesia was maintained with continuous intravenous administration of propofol (6-8 mg/kg/h), supplemented as needed with intramuscular injection of medetomidine (15 μg/kg). Heart rate and oxygen saturation (SpO₂) were continuously monitored. The level of anesthesia was controlled to keep SpO₂ above 95%. Body temperature was kept constant using hot water bags. Blood pressure was monitored between functional runs. Glucose-lactated Ringer’s solution was given intravenously (5 mL/kg/h) throughout the experiment (for details, see Supplementary Methods). Stimulation and reference electrodes were connected to a programmable constant current stimulator (SEN-7103; Nihon Khoden) controlled by a desktop computer.

**Figure 1.** Design of EM and fMRI experiments. (a) A sagittal MRI image showing placement of the stimulation electrode. Ele, stimulation electrode. c.s., central sulcus. i.p.s., intraparietal sulcus. Left, anterior. Right, posterior. Top, dorsal. Bottom, ventral. (b) Design of functional runs. Yellow symbols indicate EM blocks. (c) 210-ms pulse trains. (d) Biphasic pulses used for stimulation.
computer running presentation software (Neurobehavioral Systems) to synchronize fMRI scans and EM.

Functional runs for EM-fMRI in the present study had a standard block design similar to what have been used in previous studies using EM-fMRI (Ekstrom et al. 2008; Moeller et al. 2008; Fig. 1b). Parameters for electric pulse trains were also chosen based on previous studies (Tolias et al. 2005; Ekstrom et al. 2008; Moeller et al. 2008). Each functional run consisted of 9 rest blocks interleaved by 8 EM blocks (30 s each). During each EM block, 210-ms electrical pulse trains were delivered at a frequency of 1 Hz (Fig. 1c). Each pulse train was composed of biphasic current pulses (3.5 Hz) delivered in a monopolar configuration. Each electric pulse consisted of 200 μs of negative phase followed by 200 μs of positive phase with phase separation of 100 μs (Fig. 1d). Current amplitude for EM was also chosen based on previous studies using EM-fMRI (Tolias et al. 2005; Ekstrom et al. 2008; Field et al. 2008; Moeller et al. 2008; Canals et al. 2009). These previous studies used large current amplitudes (typically over 300 μA) to evoke reliable and detectable BOLD responses, particularly at distant brain areas, both in rats (Angenstein et al. 2007; Canals et al. 2009) and in monkeys (Tolias et al. 2005; Field et al. 2008; Moeller et al. 2008). In the preliminary experiments, where we extensively varied current amplitude, we also found a current amplitude of 300 μA or larger to be appropriate for obtaining robust BOLD responses to EM (Supplementary Fig. 3a,b). Additionally, we observed that BOLD responses to EM reached saturation at current amplitude ~500 μA (Supplementary Fig. 3c,d). Therefore, we typically set the current amplitude for EM at 500 μA.

Functional Connectivity–Functional Magnetic Resonance Imaging

As in previous studies (Vincent et al. 2007; Pawela et al. 2008), we used anesthetized monkeys (using the same protocol as in EM-fMRI) to measure infC. fc-fMRI sessions were conducted using a protocol similar to the EM-fMRI sessions, except for the electrode insertion and EM during the functional scans. All the anesthetics and imaging procedures were identical to those used in EM-fMRI. fc-fMRI data were acquired at least 2 h after the induction of anesthesia.

Data Analysis for EM-fMRI (General Linear Model)

Image data were analyzed with SPM2 (http://www.fil.ion.ucl.ac.uk/spm) and in-house software written in MATLAB (MathWorks). A high-resolution T1-weighted anatomical image was rotated and registered to bicommissural space to obtain a 3D template image for each monkey (Koyama et al. 2004). Functional images were realigned and coregistered to the template image, with interpolation to a 1 mm isotropic space. Images were then smoothed with a Gaussian kernel (1.5 mm full-width at half-maximum [FWHM]). EM-activation maps (t-score maps) were computed by voxel-wise statistical analyses based on the general linear model (GLM), implemented in SPM2. emEC was defined as the strength of connectivity, as probed by EM, between a particular voxel and the location of S1 being stimulated. We used beta value obtained from GLM in each voxel analysis as emEC of the voxel. The beta value was used to measure the magnitude of the response to EM. It should be noted that, the term effective connectivity, in this study, is used to describe the connectivity probed by electrical stimulation (Massimini et al. 2005; Driver et al. 2009; Morishima et al. 2009) and not the connectivity assessed mathematically under task performance (e.g., Friston and Buchel 2000). To obtain the summary activation map and lists of activation foci for each monkey (Supplementary Fig. 2 and Supplementary Tables 1 and 2), multiple sessions were combined and treated as fixed effects in the analyses. The significance level of activation in the summary map was set at $P \leq 0.05$ corrected for multiple comparisons by family-wise error, implemented in SPM2. Brain regions were labeled by referring to the atlas of Saleem and Logothetis (2007).

Data Analysis for fc-fMRI

In addition to standard preprocessing steps as described above, functional data underwent several additional preprocessing steps for intrinsic correlation analyses. For this, we followed standard procedures (Van Dijk et al. 2010): first, data were temporally band-pass filtered to retain low-frequency components (0.008 Hz < f < 0.08 Hz); second, spurious variance was removed from the time series by regression of 6 parameters obtained by rigid-body correction of head motion along with temporal derivatives and the whole-brain signal averaged over the region including the gray/white matters, the subcortical areas and the ventricles (total of 13 nuisance regressors). After preprocessing, data from each of the 8 fc-fMRI sessions was assigned to 1 of the 8 EM-activation maps (for details about the pairing between sessions, see Supplementary Table 3). For a given EM-activation map, the peak of activation located in S1 was extracted and used as the center of 4 mm-diameter spherical seed ROI FC-correlation maps (r map) were then computed by correlating a mean signal time course extracted from the seed ROI against signal time courses of all the other voxels in the brain. As in emEC, infC was defined so as to measure the strength of spontaneous correlation between a particular voxel and the location of the seed ROI and was given by Fisher’s $z$-transformed correlation coefficient (Vincent et al. 2007; Honey et al. 2009). $z$-transformation was used so that infC reflects the strength of spontaneous correlation more linearly at high $r$ values.

Analysis of Connectivity Maps

For the analysis of correlation between emEC and infC across sessions, correlation coefficients of the pairs of maps were calculated across all the individual voxels in each hemisphere (with the exception of voxels within 5 mm from the seed ROI used to calculate infC). Note that the same exclusion mask was applied to EM-fMRI data and fc-fMRI data when comparing emEC and infC). The radius of the exclusion mask was set to that of the seed ROI plus twice the size of Gaussian spatial filter (2007; Honey et al. 2009). The radius of the exclusion mask was approximately 1.7 times larger than the FWHM of the spatial profile of infC (6.0 ± 1.5 mm; Supplementary Fig. 4; for details, see Supplementary Methods). To investigate the correlation between emEC and infC in stimulated or nonstimulated hemisphere, voxel-by-voxel correlation of emEC and infC maps was calculated for each hemisphere. Obtained correlation coefficient was then converted to normalized correlation (NC) by applying Fisher’s $z$ transformation. Difference of NC in the stimulated and nonstimulated hemispheres was assessed using the paired $t$-test. Interhemispheric symmetry (IHS) of the connectivity maps was defined as the slope of a linear regression line fitted to a scatter plot of ipsilateral (i.e., stimulated) and contralateral (i.e., nonstimulated) hemispheres using the least square method. Voxels within 5 mm from the center of the seed ROI used to calculate FC-correlation maps were discarded in the fitting process in order to avoid contamination by artificial correlation. We used the slope of the regression line instead of the correlation coefficient to take into account the similarity of the absolute magnitude of connectivity values in the 2 hemispheres. In the across session analyses of connectivity maps, a map of emEC in a session containing less than 6 runs was discarded along with its corresponding inFC map (for details, see Supplementary Table 3). Statistical tests were conducted using Statistics Toolbox in MATLAB (MathWorks).

Results

Mapping of Electrically Evoked Activation in the Somatosensory System

Using lightly anesthetized macaque monkeys, we inserted a stimulation microelectrode into S1 located just posterior to the central sulcus (Fig. 1a) and conducted simultaneous EM and fMRI experiments. Figure 2a shows a representative EM-activation map obtained from a single session in a monkey whose right S1 was stimulated. With current amplitude of 500 μA, focal increases in BOLD activity were observed at the site of stimulation (right S1) and several (predominantly ipsilateral) brain regions known to have connections with S1 (area 5 [A5],
the secondary somatosensory cortex [S2], area 7 [A7], thalamus [Thal], putamen [Put], caudate nucleus [Cd], and ventral premotor area [F5]) (Jones et al. 1977; Burton and Fabri 1995; Burton et al. 1995; Tanne-Gariepy et al. 2002). BOLD signal time courses in these areas showed clear increases in the activity that was time locked to the delivery of EM (Fig. 2).

Examples from other sessions and monkeys confirmed the reproducibility of the pattern of activation as described above, including activation in the cerebellum. Activation in the cerebellum suggests polysynaptic propagation of the EM-induced activation (Supplementary Figs. 5–7; for lists of activation foci, see also Supplementary Tables 1 and 2). The primary motor cortex was weakly activated, though the peak of activation was not located in the area (Supplementary Fig. 8; see also Supplementary Methods).

**Mapping of FC Correlation**

After obtaining EM-activation maps, we measured functional connectivity of spontaneous BOLD activity (fc-fMRI) using the same monkeys and the same experimental setup. FC-correlation maps were obtained by computing correlation coefficients between each voxel’s time course of BOLD fluctuations with the average time course within a seed ROI placed at the peak of S1 activation in the EM-activation maps (for details, see Materials...
and Methods). Figure 2c shows a representative FC-correlation map calculated by placing the seed ROI at the peak of S1 activation in Figure 2a. As in the EM-activation map, the FC-correlation map showed high FC correlation in several discrete brain regions whose distribution appeared similar to that of the EM activations (compare Fig. 2a,c). In fact, directly overlaying the 2 maps showed that EM activations and blobs of strong FC correlation overlapped in several brain regions such as S1, A5, A7, S2, F5, Thal, and Put (Fig. 2d). However, the 2 maps also exhibited a clear difference: While EM activations appeared largely lateralized to the stimulated hemisphere, strong FC correlations often appeared in bilateral homotopic regions, resulting in a more symmetric appearance of the FC-correlation map. This intrahemispheric similarity and interhemispheric dissimilarity of FC-correlation and EM-activation maps were also observed in different sessions and monkeys (Supplementary Figs. 5–7). In addition to these general tendencies, it should be noted that relative strength of emEC and inFC varied across cortical/subcortical areas even within the stimulated hemisphere. In particular, cortical areas showed stronger inFC than subcortical areas with comparable emEC (Supplementary Fig. 9).

Quantitative Comparison of emEC and inFC

To quantitatively examine the intrahemispheric similarity between EM-activation maps and FC-correlation maps more systematically, we calculated correlation coefficients between the 2 connectivity values across individual voxels in the brain. For the data used in Figure 2, in each voxel, we measured emEC by beta values obtained from the general linear model analysis, and inFC using Fisher’s z-transformed correlation coefficients (for details, see Materials and Methods). Figure 3a,b shows representative plots of the 2 connectivity values across all the voxels in the ipsilateral (i.e., stimulated) and contralateral (i.e., nonstimulated) hemispheres, respectively. Within the ipsilateral hemisphere, 2 types of connectivity showed a positive correlation \((R = 0.36; P < 0.0001, t\text{-test corrected for multiple comparison by Bonferroni’s method for 16 hemispheres; Fig. 3a})\). In contrast to the ipsilateral hemisphere, correlation in the contralateral hemisphere was relatively low \((R = 0.10; P < 0.0001, t\text{-test corrected for multiple comparison by Bonferroni’s method; Fig. 3b})\) and was significantly less than that in the ipsilateral hemisphere \((Z\text{-test, } P < 0.0001)\). Analysis across sessions and monkeys confirmed that the correlation between the 2 connectivity values was significantly stronger in the ipsilateral hemisphere than in the contralateral hemisphere (paired \(t\text{-test, } P < 0.0005;\) Fig. 3c). The difference was also significant even when a nonparametric analysis was used (Wilcoxon’s signed rank test, \(P < 0.033\)) and did not depend on the specific pairings between EM-fMRI and fc-fMRI data sets (Supplementary Fig. 10). A similar trend could also be seen when the data from each monkey was analyzed separately (Supplementary Fig. 11a,b). Furthermore, control experiments in which EM-fMRI and fc-fMRI were conducted within the same session revealed that the presence or absence of the microelectrode during fc-fMRI did not affect the results (Supplementary Fig. 14a,b; see also Supplementary Methods). In the subsequent analyses, we examined the feature that characterized the difference between the 2 types of connectivity.

Persistent Asymmetry of emEC across Different Current Amplitudes

One feature that clearly differentiated between the EM-activation map and the FC-correlation map was the IHS of the 2 maps. Whereas the EM-activation maps appeared asymmetric, the FC-correlation maps appeared symmetric due to strong FC correlation in bilateral homotopic regions (Fig. 2a,c). To confirm that the interhemispheric asymmetry is a robust feature of emEC that is independent of the specific current amplitude used for EM, we conducted EM and fMRI using a range of current amplitudes. Figure 4 shows representative maps of emEC obtained using the current amplitude of 250 µA (top), 500 µA (middle), and 750 µA (bottom). The asymmetric pattern of emEC was consistently found across 3-fold change in the current amplitude. Note that cerebellar activation was present at all amplitudes (see \(Z = -3\) in Fig. 4), indicating that the amplitude range tested was above the threshold for evoking polysynaptic activations. Examples in other sessions and monkeys confirmed the reproducibility of this asymmetric pattern (Supplementary Fig. 12) further suggesting that, at sufficiently high current amplitudes, the asymmetric pattern of emEC does not depend on the current amplitude used for EM. The asymmetric pattern was also seen in BOLD signal correlation in the presence of EM at a high current amplitude (500 µA; Supplementary Fig. 13). Given these results, we proceeded to further analyze the IHS of inFC and emEC more quantitatively and showed that this feature indeed differentiated 2 types of connectivity.

Figure 3. Correlation of emEC and inFC. (a) Scatter plot of emEC against inFC in ipsilateral hemisphere (M1, EM session 2 and FC session 2). (b) The same scatter plot for the contralateral hemisphere (M1, EM session 2 and FC session 2). (c) Mean correlation of emEC and inFC in each hemisphere across sessions. \(n = 7\). *\(P < 0.005\) (paired \(t\text{-test})\). Error bars, standard errors (SE).
Quantitative Comparison of Interhemispheric Symmetry

To compare connectivity values across 2 hemispheres, each voxel in 1 hemisphere was paired with a voxel that takes the left–right symmetric position in the contralateral hemisphere (i.e. voxel(x,y,z) was paired with voxel(-x,y,z)). Figure 5a,b shows the relationships of the connectivity values across 2 hemispheres over all the voxel pairs (except for voxels near the seed ROI, for detail, see Materials and Methods) for emEC and inFC, respectively, for the representative example used in Figure 2. In the case of emEC, in accord with the asymmetric appearance of the EM-activation map, the scatter plot did not show apparent relationship between ipsilateral and contralateral connectivity values (Fig. 5a). On the other hand, in the case of inFC, in accord with the bilateral pattern of the FC-correlation map, the scatter plot showed a clear relationship between ipsilateral and contralateral connectivity values (Fig. 5b). For a quantitative comparison, we measured IHS using the slopes of the linear regression lines fitted to the scatter plots (dotted lines in Fig. 5a,b; slope of the regression line was used to take into account the similarity in absolute magnitude, for detail, see Materials and Methods). In accord with the more symmetric appearance of the FC-correlation map compared with the EM-activation map (Fig. 1c,a, respectively), the corresponding IHS was significantly larger for inFC than that for emEC (IHS = 0.53 and 0.083, respectively; t-test, P < 0.0001). We next examined the reproducibility of these results across sessions and different experimental conditions (e.g., current amplitude). IHS for inFC was significantly larger than for emEC at all different current amplitudes tested (t-test corrected for multiple comparisons using Bonferroni’s method, P < 0.02; Fig. 5c). In contrast, no statistically significant difference in IHS was found among emECs obtained at different current amplitudes (P > 0.6; Fig. 5c). A similar trend was observed when the data from each monkey was analyzed separately (Supplementary Fig. 11c,d) and also in the control experiment in which EM--fMRI and fc--fMRI were conducted within the same session (Supplementary Fig. 14a,c). These results demonstrated that IHS was clearly differentiated in inFC and emEC, suggesting that the symmetric pattern of inFC may be a unique feature arising from network-level interactions that are not reflected in emEC.

Discussion

In the present study, we directly compared intrinsic functional connectivity from S1 (inFC) and effective connectivity from S1 measured by EM (emEC) in anesthetized macaque monkeys. The results revealed that inFC and emEC overlapped and exhibited a significant positive correlation within the hemisphere ipsilateral to the site of stimulation (or the seed ROI). On the other hand, in the contralateral hemisphere, inFC and emEC did not overlap and showed a significantly weaker correlation compared with the ipsilateral hemisphere. Quantification of IHS revealed significant differences between inFC and emEC.

Previous studies have reported a general correspondence between emEC/inFC and underlying anatomical connections. For emEC, EM--fMRI in V1 and frontal eye field of macaque monkeys revealed EM-evoked activations in a manner consistent with well-known corticocortical connections (Tolias et al. 2005; Ekstrom et al. 2008). Similarly for inFC, Vincent et al. (2007), using isoflurane anesthetized macaque monkeys, demonstrated significant overlap between known anatomical connections and inFC. However, the precise relationship between emEC and inFC is complex, in that both connectivity measures are likely to reflect polysynaptic connections rather than simple monosynaptic connections. For emEC, the effect of thalamic EM has been previously shown to propagate poly-
synaptically using EM--fMRI (Logothetis et al. 2010). For inFC, previous studies have shown that inFC can arise even without direct anatomical connections (Vincent et al. 2007; Honey et al. 2009).

In the present study, the distributions of emEC and inFC were largely in accord with the known anatomical connectivity of S1. Corticocortical connections are known to exist between S1 and ipsilateral cortical regions near the central sulcus (e.g., S1, A5; Burton and Fabri 1995) and regions near the lateral sulcus (e.g., S2, A7; Burton et al. 1995). We also found positive emEC and inFC in the ventral premotor area (F5), an area with strong inputs from S2 and A7 (mostly 7b) (Matelli et al. 1986). Neuronal activity in F5 during tactile discrimination has been reported to encode sensory information as well as motor commands after decision making (Romo et al. 2004). Romo and colleagues reported state dependence of the neuronal activity in F5 during tactile discrimination tasks. They found that neuronal activity in F5 reflected somatosensory stimulation while the monkeys actively discriminated the vibrotactile stimuli but not when the same monkeys passively received the stimuli (Hernandez et al. 2010). The same authors also reported similar state-dependent neuronal activity in the supplementary motor area (SMA) (Hernandez et al. 2002, 2010). Given these results in task-performing monkeys, it is interesting that strong emEC in F5 and lack of emEC in the SMA were found in the present study using anesthetized monkeys. It is possible that the effective connectivity between S1 and F5 under anesthesia is stronger than that in monkeys actively performing a discrimination task. This may indicate that active gating of sensory input (Seidemann et al. 1998) is abolished under anesthesia. In contrast, the effective connectivity between S1 and SMA in anesthetized monkeys may be as weak as that in monkeys passively receiving the stimuli. Alternatively, the BOLD response in F5 may reflect synaptic inputs rather than the postsynaptic firing (Logothetis et al. 2001). Task demand may provide the input necessary for translating the synaptic inputs to neuronal firing. The lack of BOLD responses in SMA may indicate the absence of synaptic inputs. The overlap of emEC and inFC found in these regions suggests that regions included in the somatosensory processing hierarchy, encompassing sensory-related (S1) to decision-related (F5) regions, are not only coupled by strong effective connectivity but are also intrinsically coordinated as a unit. It would be of great interest to see how this close match of emEC and inFC generalizes to the other systems in the brain (e.g., visual, auditory, and motor systems).

It should be noted that the spatial and anatomical specificity of emEC is limited by several technical factors. As in previous studies (Tolias et al. 2005; Angenstein et al. 2007; Sultan et al. 2007; Field et al. 2008; Moeller et al. 2008; Canals et al. 2009), EM in the present study used relatively large current amplitudes to evoke reliable BOLD responses. Although these BOLD responses are thought to be neuronal in origin (Tolias et al. 2005; Canals et al. 2009), such high current amplitude potentially produced effects that are qualitatively different from those observed using lower amplitude stimulation (e.g., polysynaptic propagation; Strick 2002). Moreover, large current amplitudes could have compromised the spatial specificity of emEC by direct spreading. Previous studies estimated the effect of electrical stimulation using amplitude over 500 μA to spread close to 1 mm in radius (Tolias et al. 2005; Tehovnik et al. 2006). This estimation suggests that our microstimulation was likely to have activated all the layers across the cortical depth and might have activated multiple areas simultaneously (e.g., areas 3 and 1). Large current amplitudes also made it difficult to dissociate orthodromic and antidromic effects. Although the threshold for antidromic stimulation is known to be substantially lower than that of orthodromic stimulation (Tehovnik 1996; Histed et al. 2009), our repetitive stimulation with high current amplitude very likely caused a significant amount of orthodromic stimulation (Jankowska et al. 1975). Nevertheless, despite these limitations, previous studies (Tolias et al. 2005; Ekstrom et al. 2008; Field et al. 2008) and the present study have successfully demonstrated a good overall correspondence between the pattern of EM-evoked activation and known anatomical connections (see above).

IHS of inFC has been reported and extensively studied by other researchers (Salvador et al. 2005; Stark et al. 2008). The presence and the strength of this interhemispheric correlation have been shown to correlate with the thickness of the corpus callosum (Johnston et al. 2008; Lowe et al. 2008). On the other hand, previous studies using (cortical) EM--fMRI in monkeys have primarily studied EM activation within the stimulated hemisphere (Tolias et al. 2005; Ekstrom et al. 2008; Moeller et al. 2008). Notably, Field et al. (2008) combined brainstem EM with fMRI in anesthetized monkeys and found widely distributed areas of EM activation across the 2 hemispheres, whose pattern agreed with bilateral connectivity of the superior colliculus. Following these previous studies, we focused on the difference in IHS of inFC and emEC to characterize the global features of the 2 types of connectivity. It should be noted that the difference in the relative strength of inFC and emEC in individual cortical/subcortical areas (supplementary Fig. 9) might also be important for understanding the mechanism creating inFC. Several studies have suggested that the spatial pattern and the strength of inFC are dependent on corticocortical connections (Koch et al. 2002; Johnston et al. 2008; Honey et al. 2009), while others have suggested the importance of subcortical inputs (Uddin et al. 2008). The present results support the former hypothesis. Moreover, some differences between emEC and inFC within the cortical regions could be due to a difference in sensitivity of emEC and inFC to the presence of direct anatomical connections. However, these interpretations should be taken with care since emEC may also be biased toward specific types of connections (Logothetis et al. 2010). Thus future studies comparing these connectivity measures (emEC and inFC) with underlying anatomical connections are necessary before firm conclusions can be drawn (Vincent et al. 2007; Honey et al. 2009).

What causes the difference in IHS of inFC and emEC? Since we made a direct comparison of inFC and emEC using identical experimental setups, we are able to exclude confounding factors such as differences between individual subjects, anesthetics protocol, imaging parameters, or spatial inhomo-geneous sensitivity of RF coils. Correlation analysis of the low-frequency BOLD signal during EM also revealed a unilateral pattern (Supplementary Fig. 13; see also Supplementary Methods), suggesting that the difference in the method of analysis (correlation or GLM) cannot explain the difference in IHS of inFC and emEC. Similarly, since both emEC and inFC are likely to reflect orthodromic and antidromic effects (see above), the interhemispheric difference is unlikely to be explained by these effects. One explanation for the observed difference is that the symmetric pattern of inFC emerges from...
complex network-level interactions (Honey et al. 2007). Recently, de Pasquale et al. (2010) reported a lack of stationary contralateral correlation in the spontaneous neuronal activity as measured by magnetoencephalography (MEG). The lack of contralateral correlation in MEG signals, as well as the lack of contralateral emEC found in the present study, may be attributed to the relatively weak efficacy of the callosal connection in the sensory cortex (Barth et al. 1994). In the same study, de Pasquale et al. (2010) also reported that the contralateral correlation in MEG signal increases transiently in short epochs and suggested that this transient epoch was the source of bilateral pattern of correlation in BOLD signal. It may be possible that inFC in our study reflects the mode of connectivity during this transient epoch in which the synchronizability of the network is high, whereas emEC mainly reflects the mode of connectivity during the stationary period. It would be of great interest to test this possibility by observing how EM interacts with spontaneous neuronal activity (Tehovnik 1996; Driver et al. 2009). EM–fMRI with EM delivered selectively in the transient period may yield emEC more similar to inFC.

An alternative but not mutually exclusive possibility is that inFC and emEC represent different types of anatomical connections. Logothetis et al. (2010) recently reported that EM may preferentially activate corticothalamiccortical connections rather than corticocortical connections. Given that inFC has been suggested to arise mainly from corticocortical connections (Johnston et al. 2008; Lowe et al. 2008), it is tempting to speculate that interhemispheric (and possibly intrahemispheric) differences arise from a difference in the type of anatomical connections preferentially reflected in inFC and emEC. We propose that local activation techniques (e.g., EM) as well as inactivation techniques combined with whole-brain imaging (e.g., cooling, Khachatryan et al. 2008; pharmacological inactivation, Rauch et al. 2008; Logothetis et al. 2010) could be useful to answer these questions. Moreover, the way in which the plastic changes in the local neuronal circuits affect inFC and emEC in the whole brain remains an important question to be addressed in future studies (Canals et al. 2008, 2009).

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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References


