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Interregional Connectivity to Primary Motor Cortex Revealed Using MRI Resting State Images

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Abstract: The topographic organization of cortical neurons is traditionally examined using histological procedures. Functional magnetic resonance imaging (fMRI) offers the potential noninvasively to detect interregional connectivity of human brain. In the brain, there is spontaneous firing of neurons even in the resting state. Such spontaneous firing will increase local blood flow, cause MRI signal fluctuations, and affect remotely located neurons through the efferent output. By calculating covariance of each voxel referenced to the time course of a selected brain region, it is possible to detect the neurons connected to the selected region. Using this covariance method, neural connectivity to primary motor cortex was assessed during a resting state in six healthy right-handed volunteers. This interregional connectivity is similar to connectivity established by other anatomical, histochemical, and physiological techniques. This method may offer in vivo noninvasive measurements of neural projections. *Hum. Brain Mapping 8:151–156, 1999.* © 1999 **Wiley-Liss, Inc.**

Key words: functional MRI; fMRI; motor cortex; connectivity; cerebral cortex; activation

INTRODUCTION

Mapping interregional neural connectivity in vivo is a fundamental neuroscientific objective and has been explored in anatomical, electrophysiological, and PET methods. Up to now, the vast majority of functional brain mapping (PET and fMRI) has been directed toward assessing the locations of brain activations during a psychological task, rather than to investigate interregional connectivity. Notably, a few studies in this area have explored interregional connectivity during active psychological tasks [Friston 1994; McIntosh and Gonzalez-Lima, 1994].

An alternative to the connectivity inferred from neuroimaging data acquired during a psychological task is connectivity inferred from data acquired during a resting state. During a resting state, there is spontaneous firing of neurons. Such spontaneous firing is always followed by regional cerebral blood flow increases [Golanov et al., 1994]. In the rat, these blood flow increases are 20% on average, with a duration of ~12 sec [Golanov et al., 1994]. Such blood flow increases change blood oxygen levels, which in turn change the blood-oxygen-level-dependent (BOLD) signal in fMRI time course [Ogawa et al., 1992]. Neural firings in a specific brain area affect remotely located neurons in other brain areas through the efferent output. By calculating covariance of each voxel referenced to the time course of a selected brain region, it is possible to detect the neurons connected to the selected region [Biswal et al., 1995]. Another newly developed alternative to connectivity inferred from task-based neuroimaging data is connectivity inferred

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from data acquired during transcranial magnetic stimulation (TMS) [Xiong et al., 1999].

Biswal et al. [1995] first demonstrated the feasibility of using fMRI to detect connections to primary motor cortex during a resting state. However, the Biswal et al. study acquired only a single image plane (10 mm) of brain, thus limiting any comparison of the detected connectivity map to connectivity inferred from other techniques. In order to verify this result and its implications more completely, we acquired several hundred seven-slice volumes (42 mm) of brain during rest and directly compared the connectivity detected by this method to that detected by anatomical, histological, and physiological methods.

MATERIALS AND METHODS

Subjects

Six right-handed males, ranging in age from 23–47 years (mean, 33.5 years) and possessing no known neurological disorders, participated in this study. Informed consent was obtained before the subject was imaged. For all studies, the head was immobilized in a closely fitted, thermally molded, plastic facial mask that was individually made for each subject. The mask minimized head movement during MRI scanning.

Paradigms

The subjects were instructed to perform two different tasks: hand movement and rest. During hand movement, the subject repetitively performed a task in which he fully opened and fully closed his right hand. During the resting condition, each subject was instructed to remain motionless, to keep his eyes closed, and to perform no behavioral task. Subjects first performed the movement task, then the resulting condition. Activation maps from the movement task were used to define representations of the right hand in the primary motor cortex. Note that the data from the functional task have no direct relation or bearing on the data acquired during the rest. As discussed in greater detail later, the two conditions could have been performed in different sessions and in any order, and the covariance analysis connectivity map is *not* derived from priming or temporally preceding brain activation.

Data acquisition

Images were acquired on an Elscint Prestige 2-T whole-body MRI scanner. Seven continuous fMRI slices were acquired in a transverse plane using a T2*-weighted gradient-echo, echo-planar-imaging (EPI) sequence with slice thickness of 6 mm (TR = 100 ms, TE = 45 ms, flip angle = 70°). The in-plane resolution for the images was 3.28×3.28 mm^2. A total of 377 images per slice was acquired with the first 40 images acquired from movement task and the rest 337 images acquired at rest. At the end of fMRI data collection, spin-echo, T1-weighted anatomical images in the same slice positions were acquired to facilitate the precise determination of the structures corresponding to the functional activation foci. The parameters for the T1-weighted images were: TR = 650 ms, TE = 12 ms, flip angle = 90°, voxel size = $1.64 \times 1.64 \times 6$ mm^3.

Image processing

The MRI images were analyzed for each individual subject. All images were assessed for subject movement and movement was corrected by use of the AIR algorithm [Woods et al.]. Of the total 377 images, the first five images were discarded because of the instability of the initial MRI signal. The following 82 images (35 images acquired from movement task, 47 images acquired at rest) were analyzed with a clustered-pixels analysis procedure [Xiong et al., 1995] to define the cortical representation for hand movement in primary motor cortex for the individual subject. A statistical parametric image (SPI) was created for each subject using a voxel-by-voxel group *t*-test. An intensity threshold $t = 2.5$ was used to produce a significant activation signal. To further eliminate random noise [Xiong et al., 1995], the suprathreshold voxels were searched and clusters with size >5 voxels, identified with a six-connectivity algorithm (pixels that share a common edge are connected), were considered to be a real fMRI signal; clusters with a size less than the specified size were considered to be noise and eliminated from the final functional image. An ROI of primary motor cortex activated by the movement task was defined. The ROI included only the voxels that were significantly activated during the movement task.

To investigate interregional connectivity, an SPI was created for each subject using a voxel-by-voxel cross-correlation analysis of the last 290 images. The time course of the ROI for the last 290 rest images was used as a reference function for cross-correlation analysis. A clustered-pixels analysis procedure similar to that described above was used to reveal significant interregional correlation. Any cluster with a correlation coefficient >0.55 and cluster size >5 was considered significant.

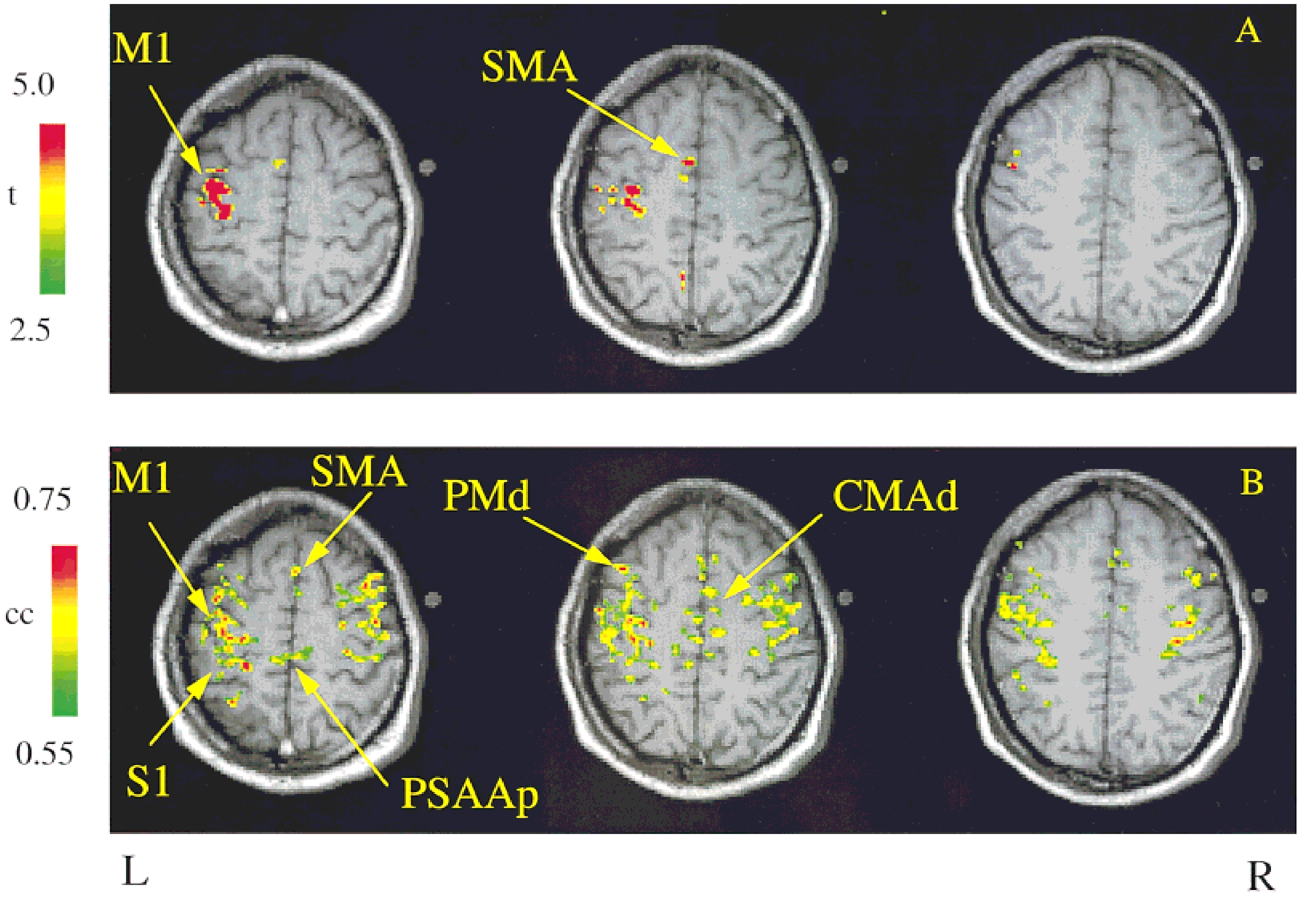

Figure 1.

Representative functional maps of an individual subject. **A.** Functional activation during simple hand movement task contrasted with the resting state. Color scale represents *t*-value. **B.** Covariance analysis of resting state MR images reveals interregional connectivity between M1 and related motor and sensory areas. Time course of M1 area was used as a reference function for the cross-correlation analysis. Color scale represents correlation coefficient (cc). PMd: dorsal premotor; CMAd: dorsal cingulate motor area; M1: primary motor area (hand); SMA: supplementary motor area; S1: primary somatosensory area; PSAAp: posterior parietal somatosensory association area.

The functional images were then linearly interpolated to the sample spatial resolution of the T1-weighted images ($1.64 \times 1.64 \times 6$ mm^3) to facilitate superimposition. The functional activation and covariation maps were displayed in corresponding color scales and were superimposed onto the T1-weighted anatomic images (gray scale). Data analysis software was programmed in MATLAB (MathWorks, Natick, MA).

RESULTS

Figure 1 shows for a typical subject the significant activations for the movement task (Fig. 1A) and the significant interregional correlations during resting (Fig. 1B). As expected, the motor task activated left primary motor cortex (M1), left primary sensory cortex (S1), and left supplementary motor area (SMA). These functional regions were identified with reference to sulcal and gyral anatomy of each brain, in accordance with generally established localization parameters.

Significant covariation was detected during the resting condition between the right hand area of primary motor cortex and other areas. This covariation implies that the areas are functionally connected. Six bilateral areas covaried with the activation in the ROI enclosing the right-hand area of the primary motor cortex. These areas were primary motor cortex, primary somatosensory cortex, SMA, dorsal premotor cortex (PMd), posterior parietal somatosensory association area (PSAAp), and dorsal cingulate motor area (CMAd). Importantly, the resting condition covariance analysis

TABLE I. Percentage of subjects activating in each brain region

Region	M1[a]	S1[b]	PMd[c]	CMAd[d]	SMA[e]	PSAAp[f]
Percentage (%)	100	100	100	67	83	67

[a] Primary motor area (hand).
[b] Primary somatosensory area.
[c] Dorsal premotor.
[d] Dorsal cingulate motor area.
[e] Supplementary motor area.
[f] Posterior parietal somatosensory association area.

shows many more activated areas than the usual task-induced activation analysis. In addition, all the areas detected are known to be closely related to primary motor cortex. Similar results were obtained for all subjects (see Table I). The percentage of subjects activating in each brain region was 86% (averaging across all areas) and ranged from 67% to 100%. Thus the connectivity maps are quite consistent among subjects.

This resting state covariance map implies neural connectivity with primary motor cortex (Fig. 2A) and can be interpreted by comparison with the connectivity established by other anatomical and physiological methods (Fig. 2B) [Dum and Strick, 1991, 1993]. These two models of connectivity are quite similar. However, whether or not there are direct connections between the areas connected by dotted arrows in Figure 2A cannot be determined from this analysis.

DISCUSSION

The present study showed that the resting-state MRI covariance analysis reveals many more activated areas

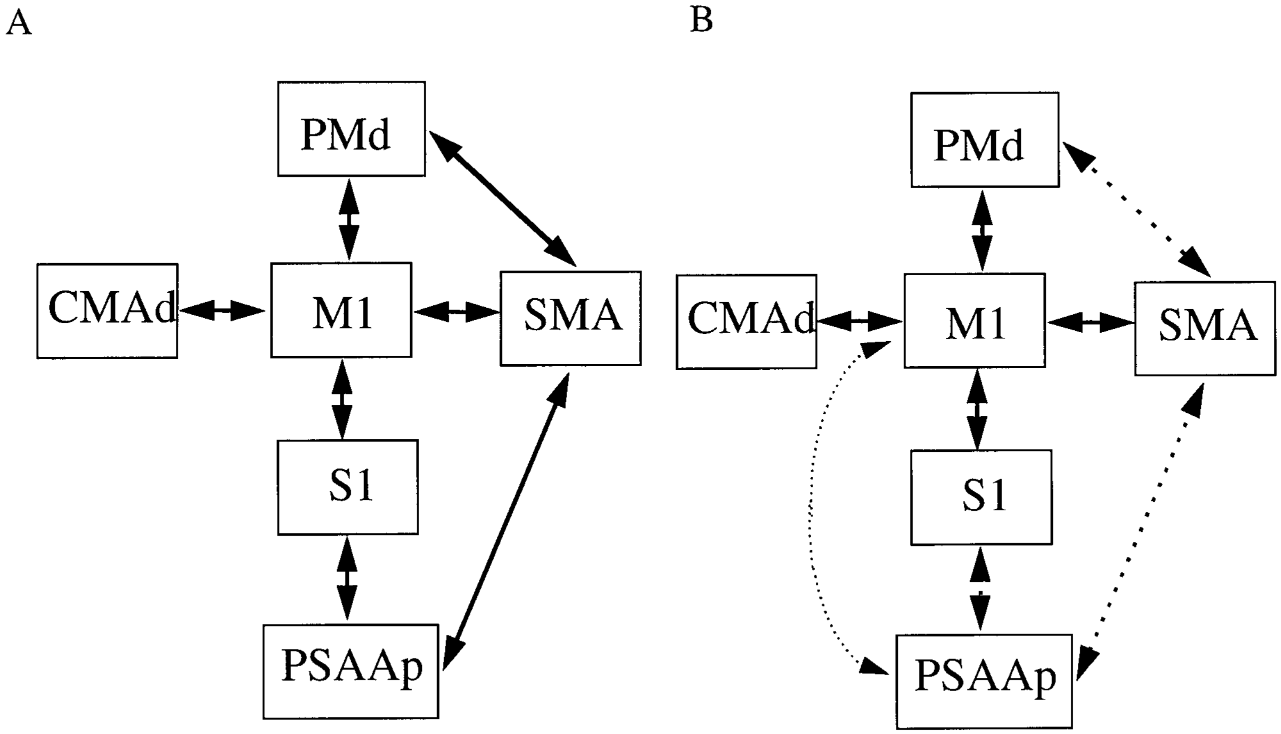

Figure 2.
Schematic presentation of interregional connectivity to primary motor area. **A.** Established anatomical connectivity to primate motor areas. **B.** Connectivity to primary motor area detected using covariance analysis of the resting state MRI. Double arrow: reciprocal neural projection; solid arrow: detected connections; dotted arrow: unconfirmed connections.

related to primary motor cortex than does the usual task-induced activation analysis. In addition, the connectivity implied by the resting-state covariance is much more similar to the connectivity established by nonneuroimaging methods. Thus our study confirms the method pioneered by Biswal et al. [1995] and extends its results. In the following discussion, we consider different strengths and limitations of this approach.

In contrast to the rest-induced map, the task-induced activation is often compared to a control state and only reveals the differences in the degree of engagement of brain tissues in different brain states (task or control). The neural activity of a particular brain region can be detected by the task-induced activation analysis only if the tissue: (1) is engaged in the performance of the particular task, (2) the degree of engagement is different between the control and task states, and (3) the differences of the degree of engagement are sufficiently large. In other words, task-induced activation may detect only a subset of a specific neural system; it may underestimate the size and number of areas involved in a task performance. However, the resting-state MRI covariance analysis detects the effects of spontaneous firing of neurons in a particular brain region on the neurons in other brain areas through the neural connections. The resting-state analysis does not require a contrast with other conditions, but allows all coactivations to emerge. It offers the possibility of more complete detection of specific neural systems. In short, whereas the task-induced activation analysis tends to detect the neural systems used in the performance of a particular task, the resting-state covariance analysis is more likely to detect all neural networks closely related to a specific brain region.

The covariance analysis of task-induced signal change also can be used to infer ‘‘functional’’ or ‘‘effective’’ connectivity of different brain regions [Friston, 1994]. Significantly activated brain regions that covary with one another are interpreted as functionally connected. This ‘‘task-driven’’ connectivity map is task-dependent. It may or may not reflect true anatomical connectivity. To take an extreme and clear case of this difficulty, when there is simultaneous stimulation during a task with both visual and auditory components, early visual and auditory areas will be co-active and the task-induced covariance analysis will falsely imply connections between the visual and auditory areas. Much more subtle versions of this difficulty could be present in actual task-induced analysis.

The resting state covariance analysis is a ‘‘task-free’’ technique in that it requires no explicit well-defined behavioral or physiological task. Of course, during a ‘‘rest’’ condition, it is likely that a wide variety of cognitive and sensory processes are activated in an unfocused chaotic sequence. When such neural activity during rest is averaged across subjects, it would likely be unbiased by focused task activations. However, in single subject data, it is possible that the mental activity during the resting state would be more systematic and would introduce an unidentified, uncontrolled ‘‘task bias’’ into the neural activations. An experimenter should be able to reduce the likelihood of such bias by refining the subject’s instructions during rest so as to minimize self-tasked, systematic mental processes. Nonetheless, in general, the connectivity map revealed by the resting state covariance analysis should not be confounded by a specific constraints of a task and should represent direct or indirect anatomical connections between brain tissues.

Thus the special contribution of our technique is that the coupling among brain areas is revealed in a way that does not depend upon cognitive or sensory motor brain state. By analyzing these spontaneous covariances, the ensuing patterns of functional connectivity are, therefore, less context-sensitive than equivalent measures obtained during activation studies. This may be very important given the context-sensitive and modulatory aspects of functional and effectively connectivity that render connectivity measurements like these very dependent upon the prevailing experimental conditions or task used. By examining spontaneous correlations, we discount this task-dependent component in a way that may bring our measurements closer to the patterns of connections defined by the anatomical infrastructure.

In the present study, each subject performed the hand movement task prior to the resting state task. However, we believe that the significantly detected activity in the subsequent resting state does not reflect a priming or carryover effect caused by the preceding motor task. First, a priming effect would be expected to decay over time; thus the activity during the subsequent resting state should be less than during the prior motor task. This prediction is clearly contradicted by our results in which more areas are revealed to covary with M1 hand region in the resting state. Second, subjects in the Biswal et al. [1995] study performed the motor task after the resting state and the resulting image data are similar to the data from our study in which subjects performed the tasks in the reverse order. Thus the order of the two tasks does not determine the detected connectivity maps. Third, when expectation during the resting state in different groups of subjects is manipulated by the experimenter to anticipate different subsequent psychological tasks, the connectivity maps of different groups are not different [Biswal et al., 1995]. Thus psychological expectation, which could lead subjects to perform covert tasks during the resting state and thereby activate the neural systems to be used in the expected tasks, does not appear to affect the acquired connectivity maps.

Rest-based interregional connectivity has so far proven to be similar to connectivity established by other anatomical, histochemical, and physiological techniques. It detects a more complete and more accurate map of human connectivity than does the task-driven analysis. The connectivity obtained by this method should improve interpretation and modeling of human brain activations during psychological task performance. This method may also offer in vivo noninvasive measurements of neural projections. Further research is required to determine how this connectivity compares in close detail with that derived from other methods, including task-induced states, structural equation modeling, and nonimaging techniques.

One limitation of the resting-state covariance analysis is that it cannot reveal the direction of neuron projections. Similar connectivity maps will be obtained no matter whether neurons are projecting to or projecting from a specific brain region. Another limitation is its relatively low spatial resolution. The typical resolution of current EPI fMRI is ~3 mm (in-plane resolution). This resolution is far lower than that of some nonimaging techniques, which can record the firing of single neuron. Moreover, it is not clear that the degree of directness of a connection could be assessed by the resting state covariance analysis. Our results showed that some indirect connections (e.g., those between M1 and PSAAp) can be detected. Further studies are required to clarify this issue.

CONCLUSIONS

The present study demonstrated that the interregional connectivity of human brain could be measured by covariance analysis of the resting state MRI images. The topographic organization of cerebral cortex for the motor system revealed by covariance analysis of the resting state MRI images closely resembles that detected by traditional nonimaging techniques (anatomical, histochemical, and physiological). Moreover, the resting state of covariance analysis provides more complete detection of the neural network closely interacting with primary motor cortex than did the hand movement task. The resting state covariance analysis is potentially a useful tool for in vivo measurements of cortical projections of the human brain. The present data should encourage wider use of this important new tool.

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