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MEASURING BRAIN CONNECTIVITY WITH FUNCTIONAL IMAGING AND TRANSCRANIAL MAGNETIC STIMULATION

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THE PROBLEM OF ATTRIBUTING CAUSALITY WITH OBSERVATIONAL FUNCTIONAL BRAIN IMAGING

Developments in functional imaging during the past two decades have allowed for significant advances in understanding how the brain functions at a systems, circuit, or organ level. Positron emission tomography (PET), single-photon emission computed tomography (SPECT), and blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) now allow researchers to image brain activity (usually related to oxygen or glucose use) with crisp spatial and temporal resolution. For example, fMRI can spatially resolve structures as small as 1 to 2 mm and view brain activity in time blocks as brief as 2 to 3 seconds. Although this time resolution is crude relative to the speed of neuronal activity and information flow between brain regions (on the order of milliseconds), these tools are nevertheless able to demonstrate the activity of clusters of brain cells through a sustained time domain in association with a behavior or task. Unfortunately, these slow time frames cannot image the directional flow of information through the brain, although exciting research in this area is under way. Thus, functional imaging tools alone have been limited in their ability to demonstrate how brain regions work in a coordinated and connected fashion to modulate information and regulate and produce behavior.

Therefore, *a fundamental problem with conventional functional imaging to date has been the inability to probe and understand the causal relationship between regional brain activity and behavior.* For example, if a brain region uses more glucose (fluorodeoxyglucose PET, or FDG PET) or oxygen (^{15}O PET or BOLD fMRI) while a subject performs a behavioral act, one can safely say that this regional activity *correlates with* the behavior. Most functional imaging researchers have correctly and appropriately used the term *correlate,* rather than *cause,* knowing well that the exact causal relationship of the regional activity to the behavior remains unclear after even the most fastidious study. For example, is the region producing the behavior? Or is the region trying to inhibit or modulate the behavior? Or is the region only incidentally activated as part of the neural network?

A recent advance in this field involves combining functional imaging with transcranial magnetic stimulation (TMS), a new technology that noninvasively stimulates the cortex. Used alone without brain imaging, TMS has been useful as a crude mapping tool for motor functions (1). Recently, by combining TMS with functional imaging, researchers have begun to test directly theories about how information flows within the brain (i.e., the functional connectivity of different brain regions). Thus, with this new combination of imaging and noninvasive stimulation, the field can now move a step closer to making causal statements of brain function. In this chapter, we introduce the technology of TMS and describe some of the important issues involved in integrating TMS with imaging to address brain connectivity. We conclude by reviewing the most recent studies in this new field in which researchers have combined noninvasive brain stimulation (TMS) with functional brain imaging.

TRANSCRANIAL MAGNETIC STIMULATION

Transcranial magnetic stimulation is a new method for noninvasively stimulating the brain (2,3). With TMS, a

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brief but powerful electric current is passed through a small coil of wires held against the scalp. This generates a powerful local magnetic field, which passes unimpeded through the skull and induces a weaker and somewhat less focal electric current in the brain (4–6). The highly localized TMS magnetic field typically has a strength of about 1 to 1.5 tesla (T) [about 30,000 times the earth's magnetic field, or about the same intensity as the static magnetic field used in clinical magnetic resonance imaging (MRI)] (7). Although different coil designs allow for more focal or more diffuse stimulation, current technology limits the depth of direct stimulation to just below the skull in superficial cortex. The magnetic field declines exponentially with distance from the coil. MRI techniques have enabled researchers actually to *image the magnetic field* of the TMS coil (8). Unfortunately, the actual important physiologic effects are likely a consequence of the *electric current density* and the *induced electric field in the area of cortex* (Appendix I). Current theories hold that the induced electric fields cause neuronal depolarization or changes in neuronal activity, which result in information flow and neurotransmitter release. Newer MRI sequences in development may someday soon allow us to image the electric current density directly and, by applying this technology to high-resolution structural imaging, actually image the induced electric field (D. LeBihan, *personal communication; May, 1999*).

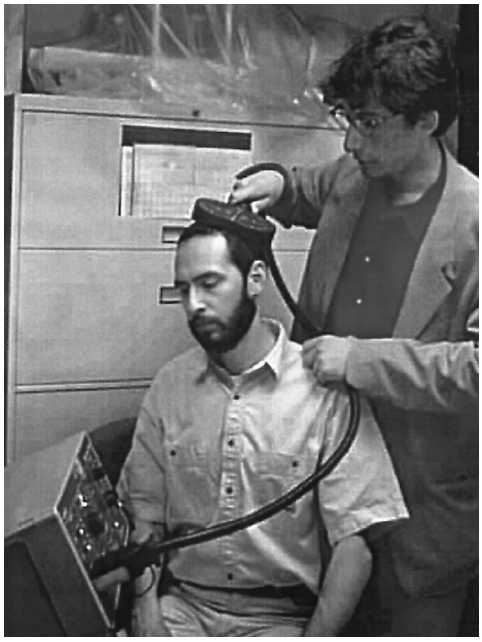


FIGURE 30.1. The chain of events by which transcranial magnetic stimulation produces changes in the brain and resulting behavior. Transcranial Magnetic Stimulation (TMS): Time-varying electrical current in a coil produces ◊ Focal 2 Tesla magnetic field passes unimpeded through skull ◊ Induces current in neurons ◊ Behavioral change.

Transcranial magnetic stimulation can be performed in outpatient laboratory settings in awake alert subjects (Fig. 30.1) and does not intentionally cause a seizure, nor does it require anesthesia (9). Subjects usually notice no adverse effects except for occasional mild headache and temporary discomfort at the site of the stimulation. Repeated rhythmic stimulation is called repetitive TMS (rTMS). Recent technologic advances have led to the development of magnetic stimulators that can repeatedly stimulate faster than once per second (1 Hz). By convention, stimulation faster than 1 Hz is called *fast rTMS*, and stimulation slower than 1 Hz is *slow rTMS.* This distinction is important because some evidence from work in animals (10) and humans (11) suggests that stimulation at different frequencies may have divergent and even antagonistic effects on neuronal activity (12,13). Importantly also, the risk for seizures in healthy adults is virtually nil with slow rTMS, and so in the United States, research with slow rTMS does not require an investigational device exemption from the Food and Drug Administration (14).

Over primary motor cortex, a TMS pulse of sufficient intensity causes movement in the opposite arm or leg (an intensity called the *motor threshold*). Similarly, a single pulse of TMS over visual cortex can produce a subjective flash of light (or phosphene). Precisely timed pulses can also interfere with, or augment, other complex tasks (see ref. 1 for review). Thus, TMS alone without imaging has been used as a relatively spatially crude mapping technique, largely over the motor cortex. rTMS at frequencies of 4 Hz or higher applied over Broca's area can cause temporary speech arrest (15). This ability to block function temporarily is frequency-dependent. It is unclear which neurons are stimulated with TMS, and whether and how this varies as a function of intensity or frequency. It is also not known how TMS causes speech arrest—whether through synaptic tetany or activation of local inhibitory interneurons.

In summary, the ability to stimulate the cortex noninvasively with TMS in an awake, alert human is an important new tool and scientific advance. At present, knowledge is limited about the physiologic and pharmacologic actions of TMS, especially as they may vary as a function of frequency, intensity, or length, in different brain regions, and in disease states versus health. Coupling TMS with imaging will likely produce new knowledge in two different areas. First, it will probably advance understanding of how TMS affects brain and *thereby refine the clinical applications* and therapeutic uses of TMS in neuropsychiatry. More importantly, from the perspective of cognitive neuroscience, combining TMS with functional imaging *will open up new avenues for the investigation of brain circuits, connectivity, and the causal chain in brain–behavior relationships* and is thus a powerful new research tool.

BACKGROUND OF THE CONCEPT OF BRAIN CONNECTIVITY AND CIRCUITS

Current Approaches to Functional Imaging Analysis and Connectivity

The first step in functional imaging is to find out which areas of the brain show activity (based on increased blood flow) when a subject performs some mental or physical task, or mentally responds to a stimulus. Conceptually, this is simple—one compares images of the brain acquired during periods when it is responding to some well-defined test stimulus with images acquired when it is performing some well-defined control task. The assumption is that the differences in the two sets of images represent the differences in brain activity during the test stimulus and during the control task. However, these are fairly subtle effects. A small change in the signal can confound the data in unknown ways. The fMRI signal is inherently noisy and often changes because of instability of the instrumentation and environmental influences on the subject . For this reason, the problem of determining the areas of the brain that are being activated by the test stimulus and assigning a probability to that determination has been extensively studied, and the field has developed commonly accepted methodologies for processing PET and fMRI data (16–22).

Determination of Regional Activation

The concept of constructing an interpolated spatial map of a statistical parameter, *significance probability mapping,* was developed in the analysis of multichannel electrophysiologic (EEG) data (23,24). In early functional imaging with PET, Fox and Mintun (16) introduced what they called *change distribution analysis,* which consisted of a subtraction of subject-averaged PET images. Present fMRI-processing methodology draws on both these ideas. In its simplest form, a pixel-by-pixel *t* test is performed by comparing the distribution of activation values for two different conditions during the course of an experiment. This gives a *t* map (i.e., an image in which each pixel represents the Student's *t* statistic for the comparison of the test condition relative to the reference condition at that location). By using the associated *p* values and the number of degrees of freedom, the *t* values can be converted to *z* values (gaussian distribution: mean 0, variance 1) to obtain *z* maps. This is the basis for statistical parametric mapping (25), formally described as the construction of spatially extended statistical processes, or maps, to test a hypothesis (usually about neurophysiology) directly. Generally based on a linear and parametric model, statistical parametric maps (SPMs) are image processes with voxel values that are, under the null hypothesis, distributed according to a known probability density function, usually gaussian. In the same way that a *t* value is interpreted by reference to Student's *t* distribution, an SPM is interpreted by referring to the probabilistic behavior of stationary gaussian fields (26) and can be used to make statistical inferences about regionally specific findings (e.g., the probability of finding an activation focus by chance). In general, SPMs characterize experimentally elicited changes in terms of (multiple) activation foci. Regions of the SPM with high or low values are interpreted as regional activations. Thus, it is possible to locate areas of the brain that are "active" during the execution of some task (i.e., the signal in those areas has a time-varying pattern that correlates with the pattern of the conditions in the experiment) (17).

Functional and Effective Brain Connectivity

Intuitively, it is common to think of *brain functional connectivity* as two or more separate anatomic areas of the brain that influence each other in the performance of some mental or physical task (e.g., recalling a name or moving a finger), or to produce a mental state (e.g., sadness). This action or state of the brain, in turn, affects the body through the somatosensory system, the sympathetic and parasympathetic nervous system, and of course the brain's neuropharmacologic/neuroendocrine hypothalamic–pituitary–adrenal system. Both in electrophysiology and functional neuroimaging, connectivity of different areas of the brain has been based on the correlation between regions. In the case of electrophysiology, this means the EEG signal (24, 27–32), and in functional neuroimaging, this means the time course of regional blood flow or glucose use (18,21, 33,34)).

Friston et al. (19) emphasize the distinction between *functional connectivity,* the temporal correlations between remote neurophysiologic events, and *effective connectivity,* the influence of one neural system on another (i.e., a functional as opposed to a causal relationship). Viewed in this way, functional connectivity is simply the observed covariance among different brain systems. It is an operational definition and says nothing about the causal relations of the observed correlations. To characterize distributed brain systems, the functional connectivity (covariance) matrix, obtained from a time series of neurophysiologic measurements, is subjected to principal component analysis (PCA) (20,35) (Appendix II). The resulting eigenimages (principal components or spatial modes) each identify a spatially distributed system, comprising regions of the brain that are jointly implicated by virtue of their functional interactions (connectivity). This analysis of neuroimaging time series is predicated on established techniques in electrophysiology (both EEG and multiunit recordings). For example, in the analysis of multichannel EEG data, the underlying spatial modes that best characterize the observed spatiotemporal

dynamics are identified with a Karhunen–Loeve expansion. Commonly, this expansion is in terms of the eigenvectors of the covariance matrix associated with the time series. The spatial modes are then identical to the principal components identified with a PCA.

Structural Equation Modeling

Principal component analysis and factor analysis approaches attempt to integrate the spatially distributed activations found in SPMs into functional systems characterized by the eigenimages or spatial modes. However, one would like to go further and explore the influence of one area on another (effective connectivity), not just the correlation (functional connectivity). Many anatomic connections are reciprocal, and simple pairwise correlations cannot resolve asymmetric influences. Structural equation modeling is an attempt to address this problem.

In describing their neural structural equation models, McIntosh and Gonzalez–Lima (21) use the terms *anatomic model* and *functional model* (36). The *anatomic model* simply represents the discrete anatomic brain regions and the neuroanatomic connections between them used in the structural equation models. These anatomic models have been derived from the observation of patients with brain lesions and from animal studies, or inferred from neuroimaging studies and the analysis of SPMs. The interregional correlations of activity are used to assign numeric weights to the connections in the anatomic model, which leads to the functional model. A *functional model,* therefore, represents the influences of regions within the model on each other through the anatomic connections, and both the magnitude and the sign of the path coefficients can be estimated. In some respects, the functional model is close to the notion of effective connectivity (20,37) because it depicts the influence of one region on another. The difference is that the influences in the functional model, unlike effective connections, are explicitly depicted as direct and indirect effects through the anatomic model. Effective connectivity, as defined by Aertsen et al. (38), resembles most closely direct effects in that an effective connection is the influence of one neural element on another irrespective of direct or indirect influences. In structural equation modeling, effective connections, or total effects, are further decomposed into direct and indirect effects by use of the anatomic model. A similar distinction can be made in covariance analysis, which is often characterized as exploratory (objective) or confirmatory (theoretical) analysis. PCA and factor analysis are essentially exploratory techniques because no constraints are placed on how the variance in the system is expressed. Structural equation modeling is typically thought of as a confirmatory approach (confirmatory factor analysis) because a causal model is usually being confirmed or disconfirmed (39).

Transcranial Magnetic Stimulation as a Probe to Alter Connectivity Networks

Although the PCA and structural equation modeling techniques are well grounded statistically and quite powerful, they cannot eliminate the possibility that apparent interactions between two regions may be a consequence of other factors. The activity of two theoretically linked regions may be modulated, either directly or via changes in neurotransmitter release, by neurologic activity in a third region that is outside of the field of view of the imaging experiment, or has not been included in the structural equation anatomic model. The two regions may also be responding to different aspects of the test stimulus, either inherently because of the nature of the task, or because of engagement of the subject in performing the task.

Since it was first developed, TMS has been used to test nerve connections, nerve excitability, and nerve conduction times. One might think of this as two anatomic areas with a single connection. Paus et al. (34) and our laboratories at the National Institute of Mental Health (40,41) and the Medical University of South Carolina (42,43) demonstrated that TMS might be combined with neuroimaging to explore the connectivity of more complex three-dimensional networks in the brain to allow the direct assessment of neural connectivity without requiring the subject to engage in any specific behavior.

Because TMS seems to have a disruptive effect in most areas of the brain, its most likely use will be to suppress the activity of a region of the brain or disrupt communication between areas. This may be done by simply applying the TMS pulse at the moment the task is performed or the stimulus is applied, and noting the changed response pattern. It may also turn out that it will be possible to apply TMS after a precisely timed delay to modulate responses (44) and so investigate brain communications at time resolutions far greater than that of the hemodynamic response, approaching that of EEG. Thus, TMS provides a noninvasive means of perturbing brain circuits both spatially and at high temporal resolution. Because it is a noncognitive stimulus, its effects are less dependent on subject engagement (''attention and performance''), and because it is a more direct and quantifiable stimulus, it more closely relates to basic neurophysiologic parameters such as nerve excitability and conduction times.

INTEGRATING TRANSCRANIAL MAGNETIC STIMULATION WITH FUNCTIONAL IMAGING: PROBLEMS AND CHALLENGES

Stimulating the brain with TMS while simultaneously imaging brain activity presents a host of unique technical problems, including (a) physically placing the coil in the scanner

and over the appropriate brain regions, (b) determining whether the TMS coil interferes with the functional image, and (c) integrating the brief time domains of TMS with the slower temporal resolution of most modern imaging tools. We discuss several of these issues and recent attempts at dealing with them.

Placement of the Coil—Structural or Functional Guidance

One of the most obvious problems in combining imaging and stimulation revolves around how to position the TMS coil over the skull. Most researchers have used either a structural or functional guidance system.

Structural Guidance

The shape of the coil determines the magnetic field in the brain, and thus the pattern of induced electric current (7, 45). For circular coils, the magnetic field is most intense near the windings. When a circular coil is placed flat against the scalp, it induces a toroidal ring of electric current in the underlying cortex that is of the same size as the coil itself but more diffuse. The electric current distributions are assumed to be broad and the effects distributed. In contrast, with figure 8 coils, a focus at the intersection of the two loops is roughly twice as intense as that obtained with a circular coil and the same current (Fig. 30.2). Although the distribution of induced current is still fairly broad, stimulation over motor cortex demonstrates that it is sufficiently focal to cause movement in one location; moving the coil less than a centimeter or even slightly changing the angle results in no movement, or movement in different muscle groups.

The coil can be positioned in several ways, based on the underlying brain structure. Perhaps the best method is to acquire a structural MRI scan of the head and then use image-guided systems to align the TMS coil precisely over a specific brain region. Several groups are exploring this option by using structural MRI and then integrating the TMS with PET (46,47). Performing the same mechanical alignment within an MRI scanner is more challenging because of the problems that arise when metal is used within a powerful magnetic field. An intermediate approach, employed by the McGill group (34), is to position the coil according to a probabilistic brain system keyed to landmarks on the subject being studied, rather than according to the subject's known anatomy as determined by MRI. This method is much easier and is adequate if one is planning on using only group statements for the statistical analysis (which requires spatially transforming the imaging data into a common brain atlas). Unfortunately, because the structural morphology of the brain and the functional location

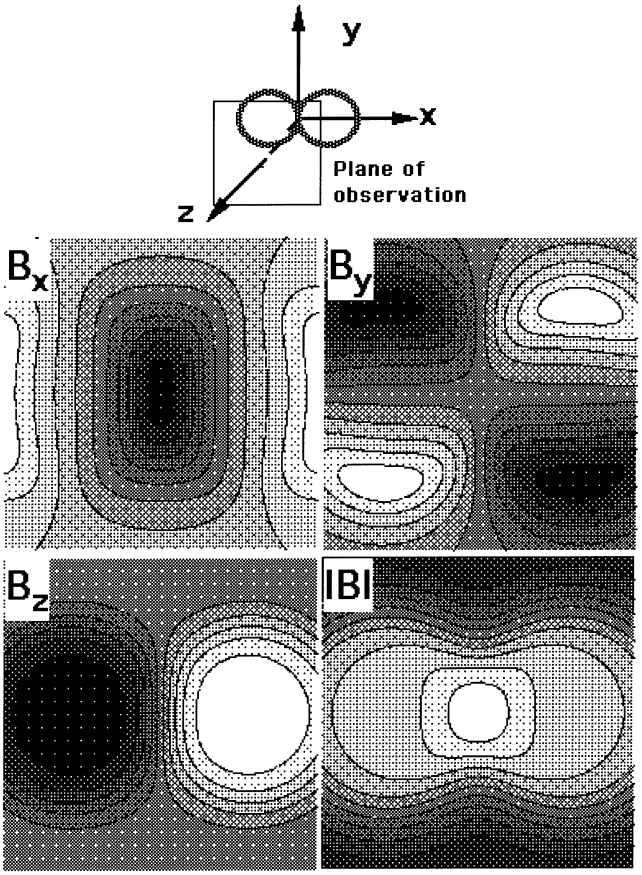


FIGURE 30.2. Magnetic field of single-turn figure 8 coil in plane parallel to and one-fourth diameter from the coil: *x, y, z* components and magnitude of field. (From Cohen LG, Roth BJ, Nilsson J, et al. Effects of coil design on delivery of focal magnetic stimulation. Technical considerations. *Electroencephalogr Clin Neurol* 1990;75:350–357, with permission.)

of behaviors vary greatly between individuals, the probabilistic method suffers from the problem that it is not certain *within an individual* whether the coil is positioned over the structure or region being studied.

The main difficulty with both image-based and structural localization is that unless one knows the exact relation of the induced currents relative to the position of the TMS coil, one really does not know where to stimulate most efficiently. The induced electric current is tissue-dependent, so it is not the same at different places on the scalp. Only if the currents had the same relation to the position of the coil, and one could use MR guidance to place the coil over the same sulcus or gyrus in a subject's brain, could one even be sure of stimulating the same structural area. However, brain conformation varies, so the induced currents might still impinge on the cerebral cortex at a different angle.

Paus and colleagues (34) approached this problem by obtaining an image volume with MR from each subject and

spatially transforming it into Talaraich space (a widely used common brain space) (48). After determining the Talairaich location shown by neuroimaging studies to correspond to a function, they performed the inverse transform back into the MR image space for each subject. Finally, using stereotactic guidance (49), they positioned the coil over this point, in effect ensuring that they were probably stimulating the functional location of the behavior in all subjects. Krings et al. (50) used a frameless stereotactic system to coregister TMS motor maps with fMRI data obtained during performance of a motor task. In two patients, they also performed direct cortical stimulation, finding good correspondence among all three methods. This probabilistic technique is more or less acceptable, depending on how consistently from one subject to the next a function is located within identifiable anatomic structures, and on how the shapes of those structures vary. Thus, this technique still entails the problem that function does not strictly map to the same location in different individuals.

Placement Based on Function

A different approach is to use TMS, electromyography, or functional imaging to determine the regions activated during a behavior and then position the coil directly over the functioning region. For behaviors like movement or phosphene production over visual cortex, one can bypass the imaging step, simply finding the scalp location with the desired behavioral effect and then keeping the TMS coil at this spot throughout an imaging study (*functional behavioral* approach to placement). Unfortunately, outside primary motor and vision areas, TMS does not produce easily viewed effects, so that this direct functional approach becomes impossible.

Despite its apparent simplicity, the *functional behavioral* approach of using elicited movement to guide coil placement to perform TMS over the motor cortex is associated with certain problems. The movement elicited by the TMS is a reassuring, if somewhat imprecise, way of being certain that one is in the correct area. However, even with comparable visible movement, one can be on one side or the other of the target area, and it is difficult to know how much or how little additional stimulation is occurring. Because this method reliably causes activation in large corticospinal circuits, we have used the functional behavioral approach for initial studies of interleaved TMS/fMRI effects over motor cortex (51,52).

Whichever method of locating the site of stimulation is used, it is important that the TMS coil be positioned accurately and repeatably, and then held securely in place so that its position relative to the brain is maintained throughout the stimulation. Each group seems to have developed its own mounting systems. We have developed a system for accurately and repeatably positioning the TMS coil within an MRI scanner (53). Both structural and functionally guided techniques have their place, and eventually systems will likely be developed for relating the two.

Interaction of the Transcranial Magnetic Stimulation Coil and Image Acquisition

Yet a different technologic problem in this new area revolves around whether and to what extent the TMS coil interferes with the functional image acquisition. Because of this concern, the early combinational studies used imaging techniques in which TMS was delivered in a location other than that where the actual brain imaging was performed (54, 55). Both FDG PET and perfusion SPECT allow one to administer TMS and deliver the radiopharmaceutical agent away from the scanner. The tracer crosses the blood–brain barrier and then settles into active regions. The subject can then be transported to the scanner for image acquisition.

Because of radiation dose limits and slow time resolution, neither of these techniques (FDG PET and perfusion SPECT) is suited for thoroughly examining circuits and behavior with the combination of TMS and imaging. For this purpose, it is much better to have the TMS coil directly within the scanner. However, one then has to understand to what extent, if any, the TMS coil interferes with the acquisition of the functional images.

In PET, groups can perform an initial transmission scan before the functional image and then subtract the minor reduction in tracer counts caused by the TMS coil. Obviously, solid core coils are not suited for this type of combinational imaging. With fMRI, the TMS coil can produce both static and dynamic artifacts. Although it may be possible to correct for the static effects, as in PET, some groups are so concerned about static artifacts that they have developed systems that quickly lift the TMS coil 2 to 3 cm from the scalp during the actual MR image acquisition following a train of pulses (56). Both mechanical and pneumatic systems have been developed to do this. Although it minimizes the potential impact of the artifact, mechanically moving the TMS coil produces shimming and alignment issues on its own and does not allow for true interleaved imaging in real time. The dynamic artifacts produced by TMS within an fMRI scanner are both more complicated and more difficult to account for (see Fig. 30.3 and ref. 53 for a full discussion). Substantial progress has been made, so that this is not a major concern.

Integration of Temporal Domains of the Scanner and Transcranial Magnetic Stimulation

The final picture produced by each of the functional imaging tools represents summed brain activity over a measure

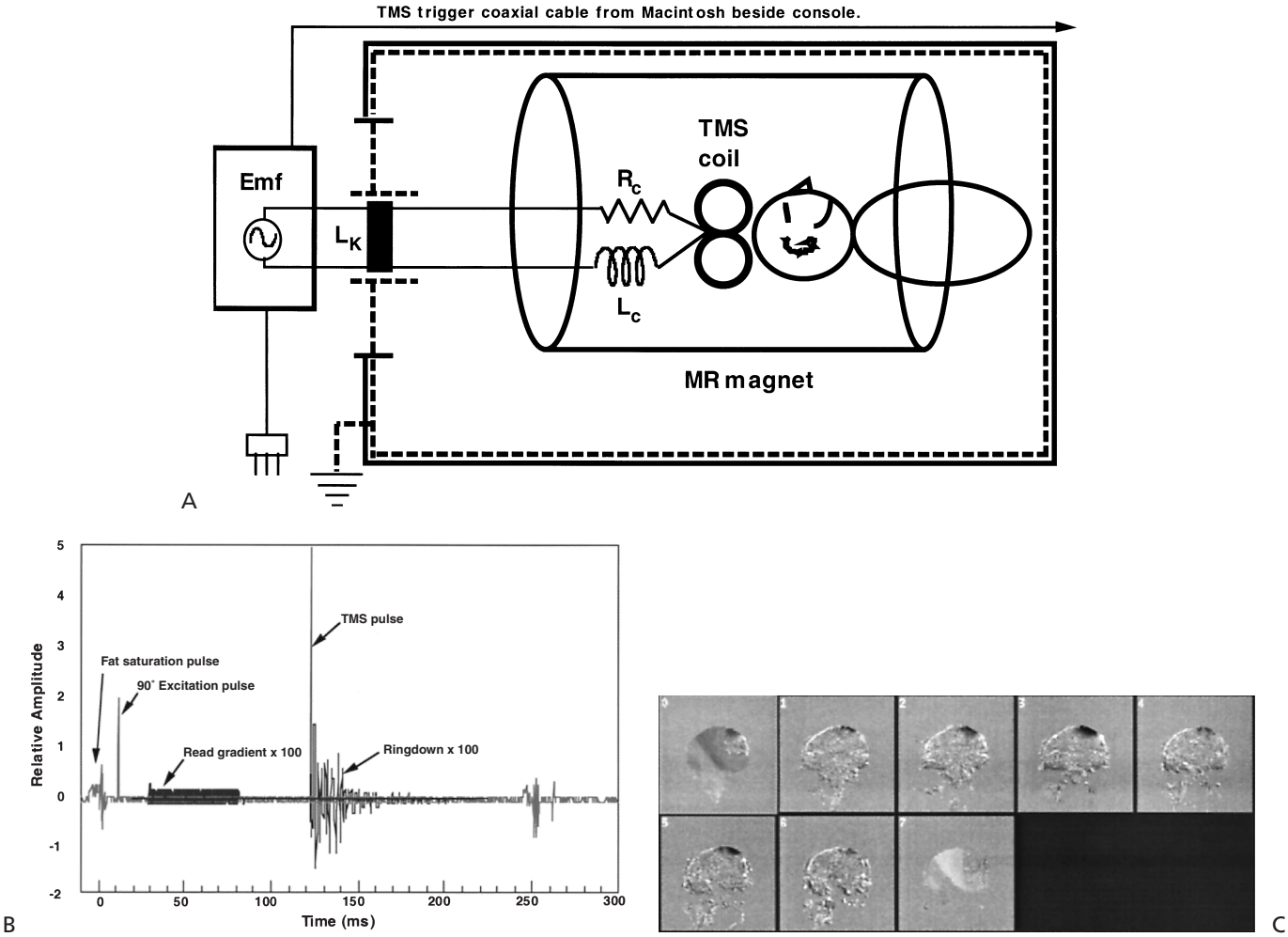


FIGURE 30.3. Researchers at the Medical University of South Carolina have recently developed the technique of performing transcranial magnetic stimulation (TMS) within the bore of a conventional 1.5-T magnetic resonance imaging scanner, the setup of which is depicted in **(A)**. This process produces dynamic TMS-induced eddy currents **(B)** and static TMS-induced eddy currents **(C)**. As seen in **(B)**, these dynamic eddy currents are approximately twice as strong as the read gradient for about 20 milliseconds, and then drop to approximately the same size as the read gradient for another 20 milliseconds. Although the major eddy currents have died out by 40 to 50 milliseconds after the TMS pulse, some longer, low-level currents are still present that cause significant image artifact **(C)**.

of time. The averaged time domain ranges from 20 to 30 minutes for FDG PET, to 40 to 60 seconds for ^{15}O PET and perfusion SPECT, to 2 to 3 seconds for BOLD fMRI, to milliseconds for EEG and electromyography. The actual TMS pulse is very brief, on the order of 300 microseconds. Thus, it is important in all combined imaging studies to understand the relationship between the TMS activity and the summed functional image. Moreover, because of the concerns of potentially causing a seizure with long trains of high-intensity, high-frequency TMS, only certain TMS parameters can be used constantly over the time domains of some forms of imaging. Unfortunately, some TMS effects, such as speech arrest, occur only with high-intensity and high-frequency stimulation.

Steady State

In this model, researchers perform TMS throughout an entire scan and then compare results with those of another scan in which all conditions are the same except for the TMS. Even with this design, and stimulation frequencies of approximately 1/s (1 Hz), most of the imaging is performed with the actual TMS machine off as a function of time.

Block Design

A different model is to scan in blocks in which periods of TMS are separated by periods of rest. An example of this is shown in Fig. 30.4, which describes the interleaved TMS/

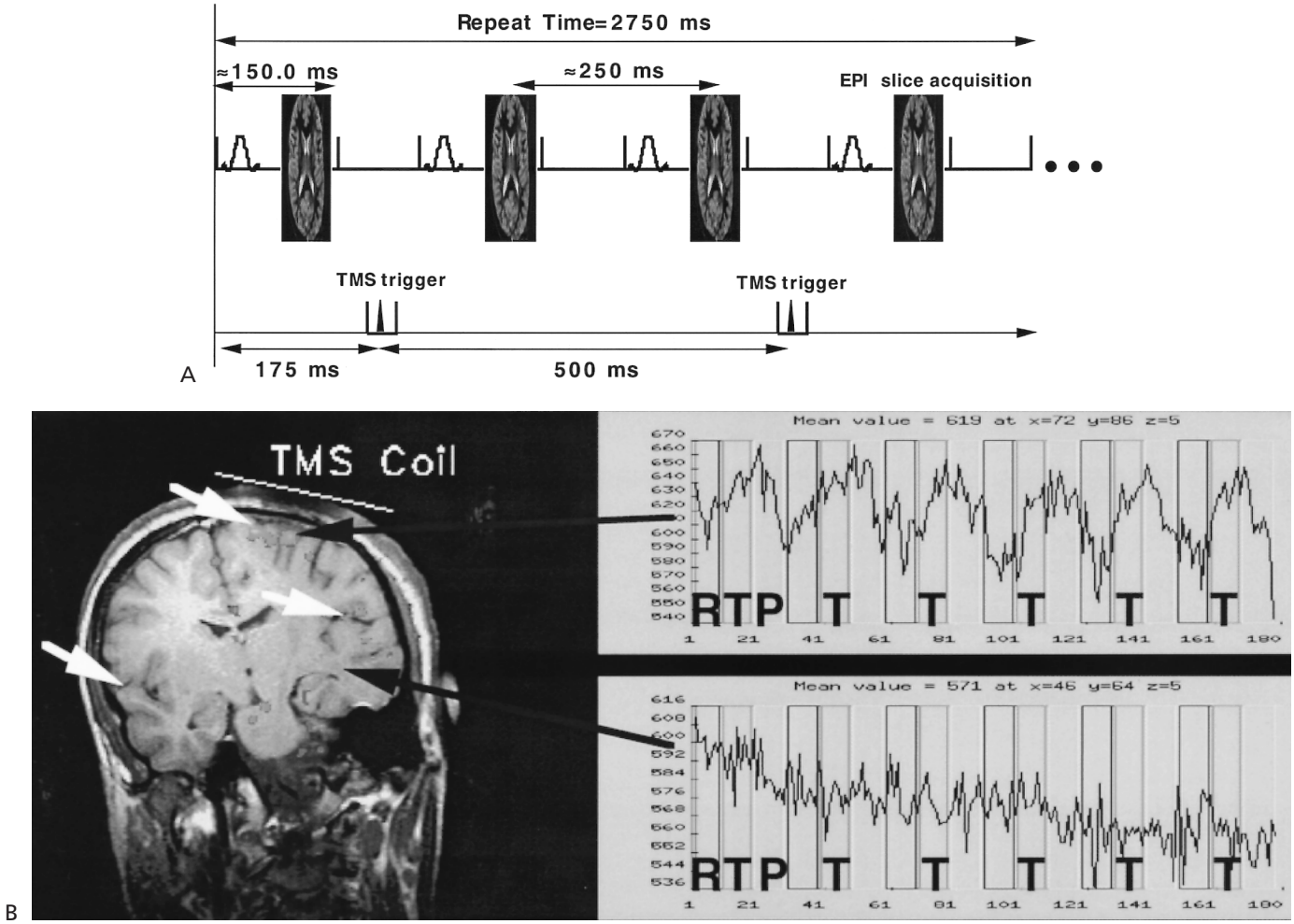


FIGURE 30.4. To perform interleaved transcranial magnetic stimulation/magnetic resonance imaging (TMS/fMRI), one must coordinate the TMS pulses with the MRI signal acquisition and interleave the two. **A:** An example of this process for a TMS rate of 1 to 2 Hz. **B:** An example of serial blood flow changes underneath the TMS coil (over left motor cortex) and in a control region. Note the increase from rest, *r*, in absolute blood oxygenation-dependent (BOLD) activity underneath the coil when it discharges at 1 Hz (task, *T*), and how it decreases afterward (post, *P*).

fMRI setup. Figure 30.5 portrays results of a recent study in which TMS was administered over motor cortex. This study showed that TMS at intensities slightly greater than motor threshold (110%) activates approximately the same number of pixels in the same region as does a volition movement (Fig. 30.5C). This study also revealed the relative magnitude of the TMS effect and the temporal relationship to changes in blood flow.

Single-Event fMRI

The method that is currently closest to the actual timing of TMS and brain events is single-event fMRI, or averaged-single-trials fMRI. With this method of scanning, images are steadily acquired at a rapid rate while the performance of a single event is rapidly interspersed. One can image the brain activity associated with a single TMS pulse by repeating the event many times and averaging the images acquired at similar times after the events, much as electrophysiologists have done with evoked responses (57). Although the BOLD response is relatively sluggish (on the order of 2 to 3 seconds), some groups are experimenting with the initial slope of the response to attempt to increase time resolution (Fig. 30.6). Applying TMS pulses to different brain regions with different interpulse interval times (milliseconds) may represent a unique way of improving the temporal resolution

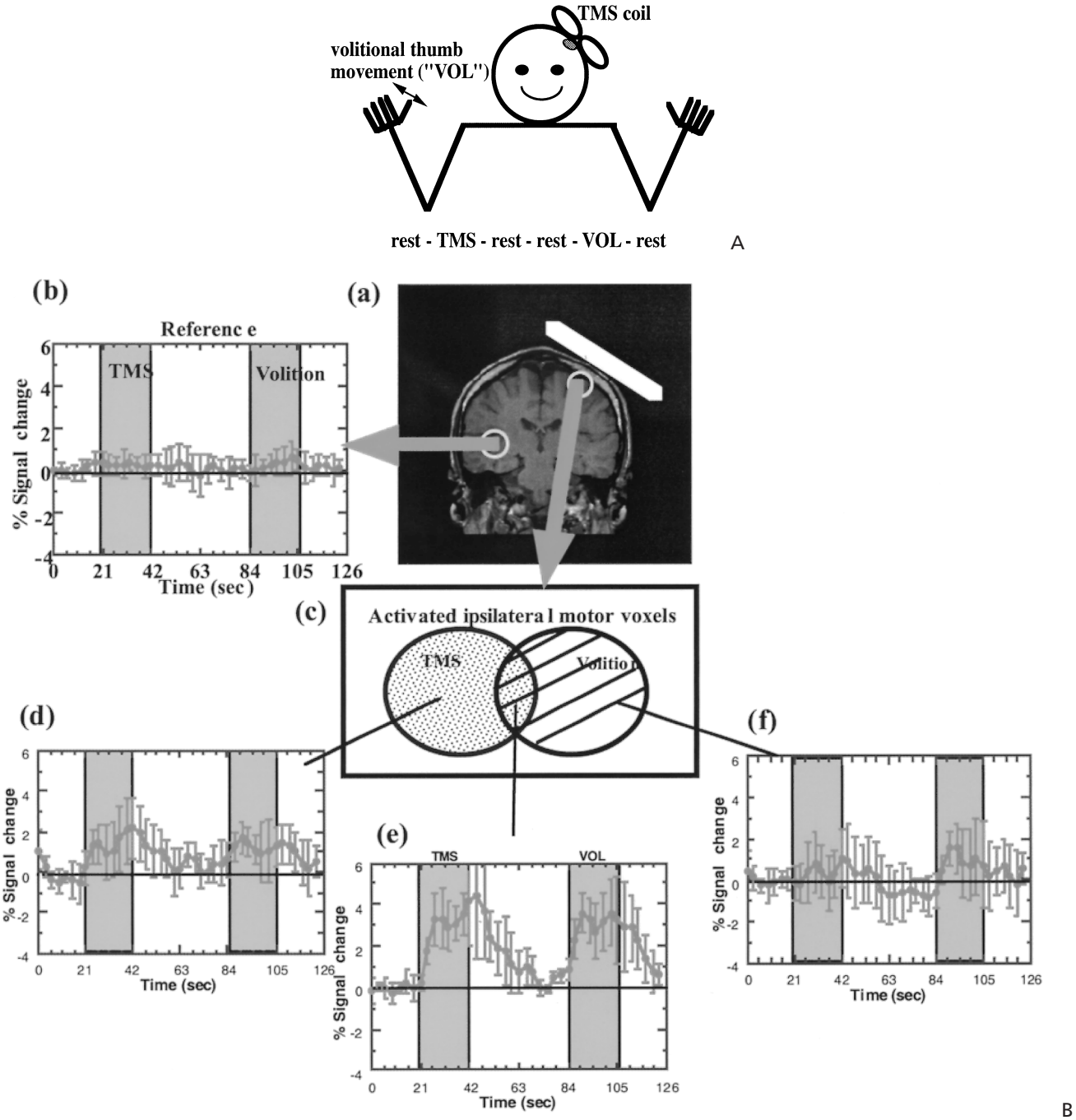
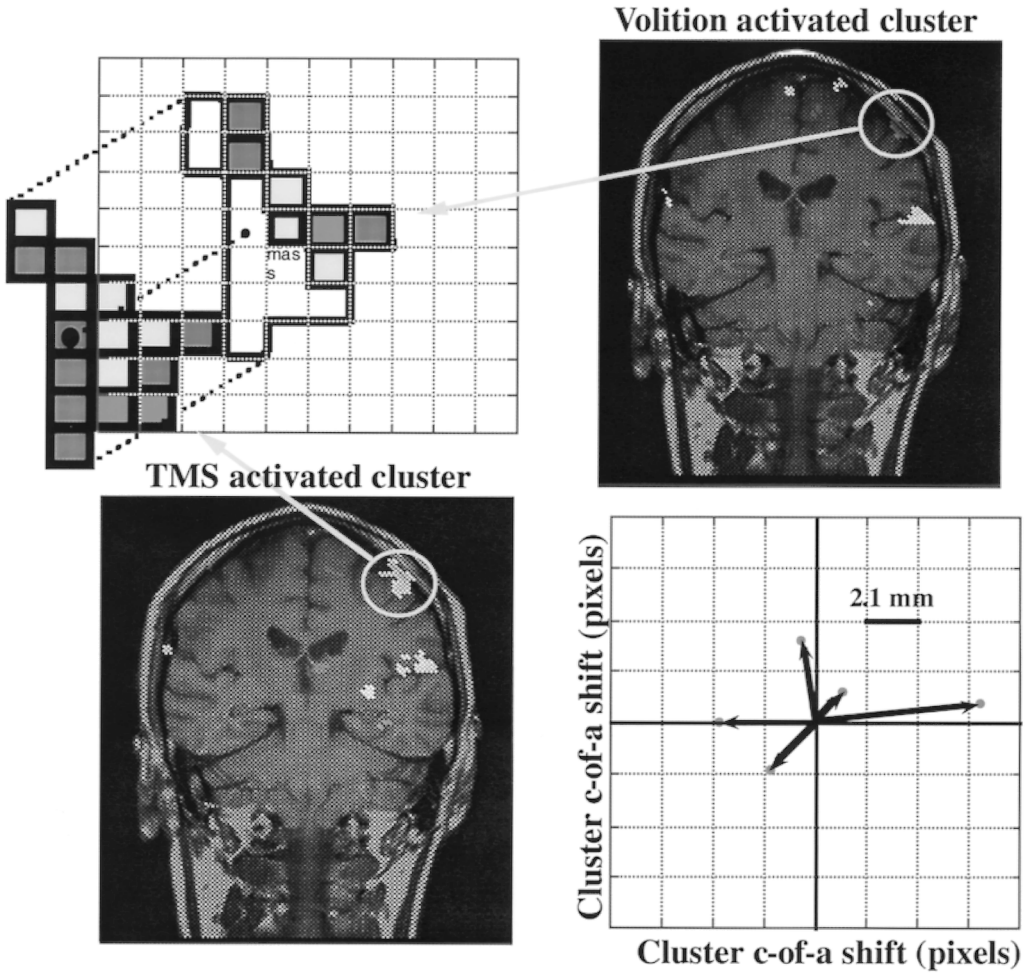
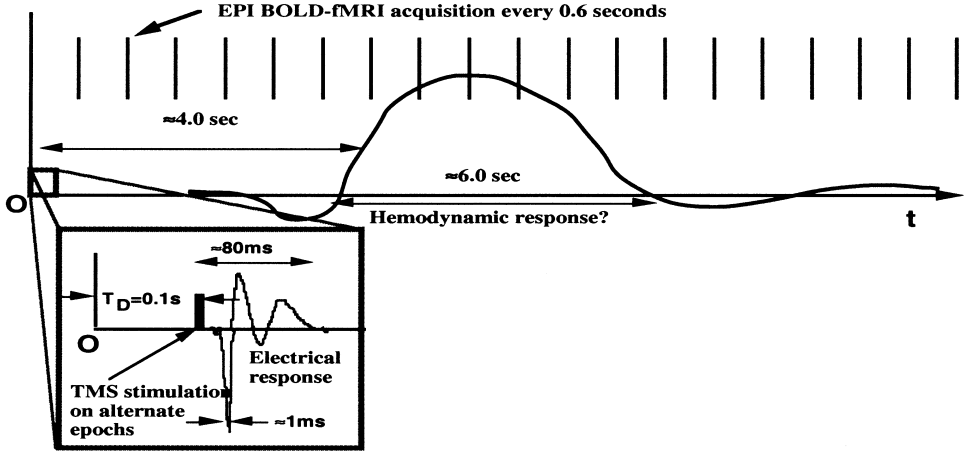


FIGURE 30.5. One of the first studies in which this interleaved technique was used attempted to detect differences between volitional and transcranial magnetic stimulation (TMS)-induced movement of the thumb. In **(A)**, the TMS device was placed over the left motor cortex of subjects, who alternately had TMS move their thumb *(TMS)* and then volitionally moved their thumb in response to a tone *(VOL).* In **(B)** are averaged group time series of brain activity during TMS, volition, or a noise control region *(upper left).* Note that for voxels that were activated in both tasks, the percentage rise in blood oxygenation-dependent (BOLD) activity does not differ from baseline. Thus, TMS produced BOLD changes that are dynamically similar to those of regular movement. *(Figure continues.)*



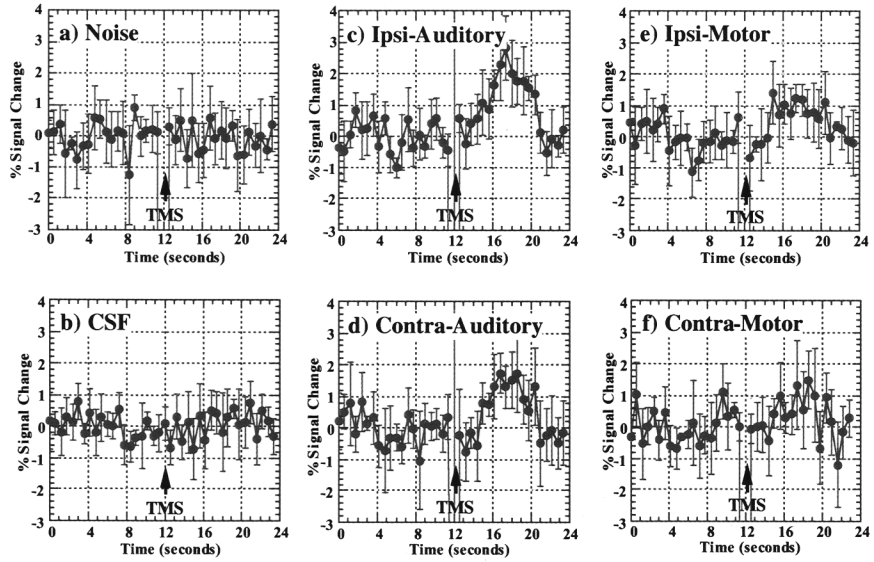
C

FIGURE 30.5. *Continued.* In **(C)**, the center of mass of the BOLD signal is virtually the same for both TMS and volition, within the limit of resolution of the magnetic resonance imaging scanner (2 mm).



A

FIGURE 30.6. One can also use an averaged-single-trials approach of transcranial magnetic stimulation (TMS) and functional magnetic resonance imaging (fMRI)—that is, one can discharge a single TMS pulse and then measure the blood oxygenation-dependent (BOLD) response *(top). (Figure continues.)*



B

FIGURE 30.6. *Continued.* The time series above show the BOLD response in a control region, for the auditory cortex, and in motor cortex. Note that a single pulse of TMS over motor cortex sufficient to cause the opposite thumb to move produces more blood flow changes in auditory cortex (caused by noise) than it does in the motor cortex under the coil.

of BOLD fMRI and of studying information flow within circuits. With further refinement, the combination of single-pulse and paired-pulse TMS and averaged-single-trials fMRI will probably be of considerable interest in *in vivo* neurophysiology.

REVIEW OF TRANSCRANIAL MAGNETIC STIMULATION FUNCTIONAL IMAGING STUDIES TO DATE

Transcranial Magnetic Stimulation Interleaved

Fluorodeoxyglucose PET

The first published combination of TMS and functional neuroimaging in real time was performed with FDG PET in a patient before and after rTMS treatment for refractory depression (54). At a separate time, these investigators also injected the glucose while the patient was intermittently stimulated at 20Hz over the left prefrontal cortex for 20 minutes. In comparison with her depressed scan at baseline, her total brain metabolism rose following weeks of TMS treatment. Also, the scan that was taken during left prefrontal TMS showed marked increases in activity, especially over the prefrontal cortex. Conclusions from this single case study are limited.

Complexity of the Issues as Demonstrated by Initial Simple Studies over Motor Cortex

A basic question for TMS and functional imaging is what happens to blood flow or activity in motor cortex while TMS is stimulating the thumb. A straightforward hypothesis would be that TMS increases blood flow in a manner similar to that produced by volitional movement. Confusion ensued when an early and still unpublished study of 1-Hz stimulation over the motor cortex for thumb showed decreased glucose uptake at the putative site of stimulation and in the contralateral motor cortex (40). Stimulation was performed at 1 Hz because FDG takes 20 minutes to settle into neurons and is thus a composite picture of brain activity over 20 minutes. This paradoxical decrease in localized brain activity both under the coil and at the mirror or contralateral site during TMS was surprising, but findings of decreased brain activity like this had been found in some electrophysiologic studies (12). The final image was a summed picture of 20 minutes of brain activity. It is likely that TMS has multiple different effects during that time—increased activity immediately with stimulation, decreases during the rest time between TMS pulses, and dynamic changes across the 20 minutes. Peter Fox and one of the chapter authors (MSG) (58) next sought to test this finding directly by using ^{15}O PET rather than FDG PET, with the TMS coil directly in the scanner. ^{15}O PET has a shorter time frame (approximately 1 minute for tracer uptake) than ^{18}FDG PET (20 to 30 minutes). Therefore, imaging with ^{15}O PET during stimulation requires that the TMS coil be placed inside the PET gantry. Using the exact same design as in the FDG study, but scanning every 10 minutes for 1 minute, we found that slow (1-Hz) rTMS over the motor cortex caused an *increase* in cerebral blood flow, although this was noted in only four subjects (58). Both of these studies used a functional behavioral placement

of the TMS coil over the optimal position for movement of the thumb and stimulated at or near the motor threshold with visual confirmation that the TMS was producing activity in the motor circuit. Thus, the results of these two initial studies were confusing and frankly contradictory.

To add even more confusion, Paus et al. (34) in the same year published a study combining ^{15}O PET and TMS. In this study, intermittent fast (10-Hz) rTMS over the frontal eye fields for 1 minute caused dose-dependent *increases* in blood flow at the stimulation site and in visual cortex. In other words, when they increased the number of 10-Hz trains within the minute, blood flow increased. Surprisingly, when the same investigators used the same rTMS parameters in the same subjects but shifted the coil to motor cortex, they found a *dose-dependent reduction in cerebral blood flow* (59). Importantly, they positioned the coil based on a probabilistic brain, and they also stimulated below motor threshold. No thumb movement occurred in these subjects.

Thus, the initial dream of using TMS and imaging to address connectivity problems in the brain has been hindered by a lack of consensus about basic imaging and TMS questions. Using yet a different technology, BOLD fMRI, our group in several studies consistently found that over much shorter time domains (7 to 30 seconds), TMS at motor threshold or above, positioned by a functional behavioral approach, consistently produced increases in blood flow at the stimulation site and in connected regions, such as the contralateral motor cortex and cerebellum (51,52). The issue now appears to be settled; the same National Institutes of Health group that found decreases with FDG PET has recently completed a more fastidious ^{15}O PET study. In this study, Speer and colleagues found dose-dependent *increases in blood flow* in motor cortex with 1-Hz TMS, as was noted in the study of Fox et al. (58) and confirmed with the BOLD fMRI technique by our laboratory (A. Speer, *personal communication; May, 2000*).

There is now a small consensus in the existing literature that blood flow increases under the motor cortex in a dose-dependent manner when the TMS coil is positioned by finding the appropriate spot for optimal thumb movement (functional behavioral technique) and stimulation is above motor threshold (and activates large excitatory neurons). When the TMS coil is positioned in this same region by a probabilistic approach, dose-dependent decreases have sometimes been found. Thus, some of the discrepancy in the literature can be explained not only by differing time domains of the imaging technologies, but also by potential differential effects caused by the method of coil placement. In this vein, using an identical study paradigm as their most recent TMS motor study, the National Institutes of Health group (Speer and colleagues) stimulated the same subjects over the prefrontal cortex, defined simply as a certain distance from the motor area (a very crude probabilistic approach, such as has been employed in many of the TMS challenge and clinical studies). Paradoxically, this group found in these same subjects *dose-dependent decreases* in blood flow over prefrontal cortex. In earlier work at 80% motor threshold, we found similar decreases in eight healthy adults when we used perfusion SPECT, with a tracer uptake time of 30 to 40 seconds, to image cerebral blood flow during fast (20-Hz) left dorsolateral prefrontal cortex rTMS (60). In comparison with a control scan with sham TMS, we found relative decreases under the coil site and in the anterior cingulate and orbitofrontal cortex. In contrast, a recent BOLD fMRI study over prefrontal cortex by our group found increases in blood flow at 120% motor threshold (61). With fMRI, one can examine individual differences, and a great deal of heterogeneity of response was noted across subjects. We are currently performing repeatability studies within subjects over time to address the inherent noise in this scanning system and the question of whether repeated TMS/fMRI studies yield consistent results.

The two most likely explanations for the opposite findings over motor and prefrontal cortex are that different brain regions react differently, or that the method of TMS coil placement matters, and that the effects of clear stimulation of large corticospinal neurons may be different from those of nonspecific stimulation of local inhibitory neurons with only probabilistic positioning. Obviously, a series of studies is needed to settle this most important issue. For example, an important next study would be to test directly the issue of blood flow as a function of functional behavioral versus probabilistic coil placement and see if functional positioning produces increases in blood flow and probabilistic placement decreases, presumably secondary to differential stimulation of excitatory versus inhibitory neurons.

BOLD fMRI

As mentioned above, the most promising, but also the most technically challenging, TMS imaging modality is a combination of TMS and fMRI. Bohning et al. (51) first demonstrated the capability of interleaving TMS and BOLD fMRI with good spatial and temporal resolution. This technique was initially thought impossible by many because of concerns about introducing a focal TMS magnetic field (1 to 2 T) inside a clinical MRI scanner of 1.5 T. Our group has found that this technique, with the right precautions, is both feasible and safe. Considerable progress has been made in devising a system for interleaving TMS with fMRI (53). Figure 30.4 shows one subject's brain with areas of TMS-induced activation superimposed in color. The time–activity curve shows the changes in BOLD signal over the course of the experiment as the TMS machine is alternately triggered at 1 Hz for 18 seconds and then turned off.

Work to date with this technique has shown that it is sensitive enough to detect subtle differences in brain blood flow response that result from minor changes in TMS inten-

sity (52). Additionally, direct comparisons of blood flow changes in motor cortex caused by TMS or volition show a surprising similarity between TMS-induced changes and those associated with normal movement (71). For example, the location of the peak blood flow change is the same for TMS and normal movement (within 2 mm). Also, stimulating at around 1 Hz and just at motor threshold activates roughly the same amount of brain tissue, and to the same degree. Thus, although many have the perception that TMS is causing supraphysiologic changes in the brain, these data imply that TMS at these parameters is acting remarkably like normal physiology.

Using Interleaved TMS/fMRI to Address Issues of Connectivity: An Initial Study

Several electrophysiologic studies have suggested that 1-Hz TMS over time domains of 3 minutes or more is inhibitory (12). To test whether this inhibitory effect occurs at time domains of several seconds, we performed TMS within an fMRI scanner and measured blood flow with the interleaved TMS/fMRI BOLD technique. Within a 1.5-T MRI scanner, five adults were stimulated by applying a figure 8 TMS coil over the left motor cortex at the optimal spot for producing movement in the contralateral (right) thumb (abductor pollicis brevis). In 21-second epochs, subjects alternated between rest and a sequential finger opposition task in their left (nondominant) hand. In alternating-movement trials, TMS was applied either at 120% motor threshold or 10% of stimulator output (below the threshold for movement) (Fig. 30.7). Coronal echo-planar BOLD images were acquired continuously throughout, interleaved so that TMS occurred 100 milliseconds after every fourth image acquisition.

With this technique and at these short time intervals, TMS did not inhibit the local or remote BOLD response during movement. In fact, TMS of the left hemisphere caused a local 1.5% increase in blood flow in addition to the 2.5% activation caused by the complex movement. We therefore concluded that the application of TMS over motor cortex for 21 seconds during a motor task-enhanced motor cortex activation does not inhibit the BOLD response. Actually imaging the remote inhibitory or modulatory effect of TMS at a different site remains for the future.

Averaged Single Trials

There is no doubt that a single TMS pulse applied to motor cortex is capable of causing a neuronal response because its consequences can be clearly seen in the form of an overt movement of the contralateral extremity. However, to date, the only functional neuroimaging technique in which the response to single-pulse TMS has been observed is EEG. Our laboratory recently sought to determine if interleaved TMS and fMRI could be used with an averaged-single-trials protocol to detect BOLD response to neuronal activation induced by a single TMS pulse, and to measure its time course.

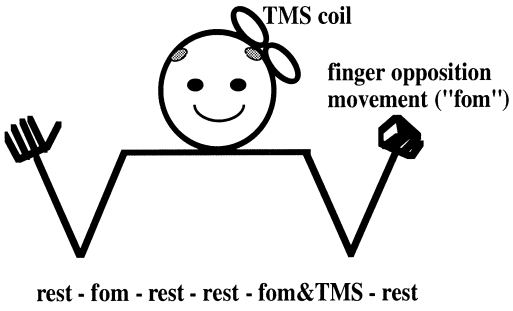
The technique is important because it allows a comparison of different TMS events by means of their associated BOLD responses. For example, with a single-event technique. it might be possible to compare different TMS intensities, or coil orientations, or single versus paired stimulation (through one coil or possibly two different coils, one conditioning coil and one test coil). Such studies could provide a bridge between electrophysiology (variation of motor evoked potential amplitudes) and fMRI (variation of BOLD response). Moreover, combining TMS with precise timing relative to a behavior with the averaged-single-trials technique would likely make it possible to image the activity of brain circuits and their connections.

In an initial study in this area, five healthy volunteers were studied with interleaved TMS/fMRI and an averaged-single-trials protocol (57). The BOLD fMRI response to single TMS pulses over the motor cortex was detectable in both ipsilateral motor cortex under the TMS coil and contralateral motor cortex, and also bilaterally in auditory cortex. The associated BOLD signal increase showed the typical fMRI hemodynamic response time course. The response of the brain to a single TMS pulse over motor cortex at 120% of the level required to induce thumb movement (1.0% to 1.5% signal increase) was comparable in both level and duration with the auditory cortex response to the sound accompanying the TMS pulse (1.5% to 2.0% signal increase) (Fig. 30.5).

Thus, ultimately, TMS combined with fMRI may allow for more exact positioning of the TMS coil, with information obtained about the magnetic field produced and also about alterations in physiology and biochemistry. Refinements of the averaged-single-trials technique and precise timing of TMS offer the potential of increasing the temporal resolution of fMRI and promoting its evolution into a tool for assessing connectivity. Much background work is needed before this combined technique can achieve its potential.

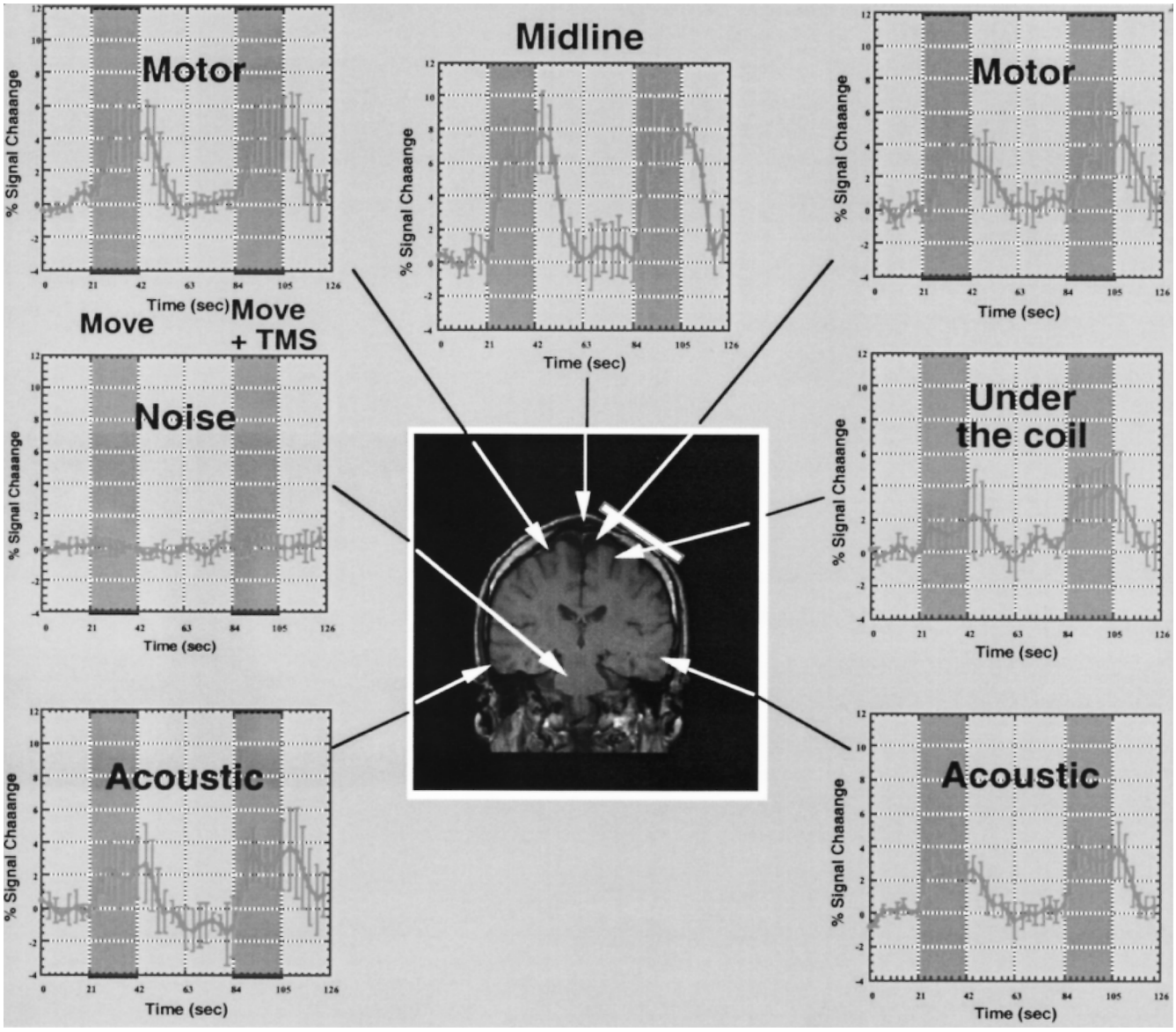
Quantitative EEG

Ilmoniemi et al. (62) have combined high-resolution quantitative EEG (qEEG) with TMS and reported regional changes in spectral content that shifted over very brief episodes of time and corresponded with known regional connections with primary motor cortex. High-resolution EEG clearly has the best temporal window of all the techniques (in the millisecond range), although the spatial resolution is poor. Unfortunately, this group in Finland is the only one to date to be able to circumvent the technical problem of recording EEG immediately after TMS and so avoid the artifact produced by the TMS pulse. This area has not advanced as rapidly as expected in the last 3 years, perhaps because of the complexity of the technique.



A

FIGURE 30.7. Several transcranial magnetic stimulation (TMS) electrophysiology studies have demonstrated that low-frequency TMS over one motor cortex can inhibit the opposite motor region. In this study, we applied TMS over the left motor cortex and had subjects perform a complex task with their nondominant (left) hand. TMS was applied on alternate epochs. We hypothesized that TMS would inhibit the blood oxygenation-dependent (BOLD) response in the right motor cortex. We did not see this. Interestingly, TMS produced an increase in BOLD response under the coil in an area of cortex that was already active. This simple study in which TMS was used to test connectivity highlights many of the issues raised in this chapter concerning how and where to apply TMS, and whether baseline activity in the underlying brain matters in terms of response.



B

Transcranial Magnetic Stimulation and Multimodal Integration

Lastly, several groups have now used TMS in a complementary way to address systems neuroscience questions. The TMS aspect of the study serves to confirm or validate a result from a purely functional imaging study. Two recent studies illustrate many of the important aspects of this type of work.

Kosslyn and colleagues (63) used TMS to investigate secondary visual cortex (area 17) and visual imagery. As a first part of this study, a traditional ^{15}O PET study was performed in subjects while they visually imagined a stimulus. As predicted from previous imaging studies in humans and animal studies, area 17 activated during this task. In a different cohort, with the use of probabilistic positioning, TMS over putative area 17 interrupted this visual imagery task. This study suffered in several respects, most notably in not knowing whether the TMS actually was applied over area 17. It nevertheless demonstrated the potential of using TMS as a convergent method of testing brain–behavior theories.

In a more elegant and rigorous application of this approach, Desmurget and colleagues (64) used TMS and imaging to test the role of the posterior parietal cortex in correcting the ongoing trajectory of movements (64). They scanned healthy subjects while they pointed to visual targets that either remained stationary or moved during saccadic eye movements. Then, using a functional image-based positioning system, they applied TMS over the left posterior parietal cortex during stimulus target presentation. The TMS disrupted the normal path corrections that occur in moving objects. Thus, in this study, TMS indicated the necessary and critical role of an area in the performance of a behavior and extended the traditional observational imaging approach.

CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

Transcranial magnetic stimulation combined with functional imaging offers the promise of a better understanding of brain circuits and the causal relationship between behaviors and activity in distributed brain regions. Several studies with a variety of imaging modalities have begun to use this approach. These studies have largely demonstrated that before the combination of TMS and imaging can be used fully, much more work is needed to improve methods. Very basic questions remain largely unexplored. These include how best to position the coil (functional behavioral versus probabilistic), how to adjust the intensity for nonmotor areas of cortex, and whether to account for differences in depth into the brain (e.g., atrophy). Additionally, a true understanding of TMS effects on the brain are still lacking, and this incomplete knowledge contributes to the lack of understanding of how best to use TMS to address systems neuroscience questions. For example, do different frequencies of stimulation produce varying effects on local metabolism, and if so, how? More complete knowledge of the local pharmacologic effects of TMS as a function of the many parameters of use would greatly advance our ability to apply TMS/imaging in neuroscience research. It is obvious that a great deal of systematic work is needed to understand this interesting tool.

However, although a better knowledge of TMS brain effects would expand and improve its use as a neuroscience tool, the ability to combine noninvasive stimulation of the brain with real-time functional imaging is an important new technique that will no doubt add to our ability to understand brain connectivity.

APPENDIX I

Determining The Appropriate Model For Calculating The Induced Electric Current In The Brain

Although, typically, the spherical model has been used, this assumes that the brain is a sphere with uniform conductivity inside spherical shells with different conductivities, corresponding to the skull and scalp. One group has gone so far as to use tissue segmentations based on MR images and estimates of gray and white matter and cerebrospinal fluid from the literature and the theoretic field of the TMS coil to perform finite element computations of the electric currents induced in individual brains (69). Unfortunately, these computations of the electric currents were performed with special field computation software and a supercomputer (69). Although the assumptions of most computational models that the brain is spherical and the cortex is an isotropic, homogeneous volume conductor are simplistic, some important observations have been made. The charge accumulation on the tissue surface tends to cancel the perpendicular component of the induced electric field, shielding the brain. This forces the resultant electric field to lie predominantly parallel to the tissue surface and fall rapidly with depth (65–68). These observations are also expected to be valid for models that more faithfully represent the actual shape and composition of the brain by treating it as a summation of finite elements. To take into account the inhomogeneous conductivity of the brain, Cerri et al. (69) used MR images to segment the brain into white matter, gray matter, and cerebrospinal fluid, and a conductivity versus gray level interpolation function derived from tissue conductivity data in the literature to obtain a three-dimensional conductivity map. They then divided the brain into discrete resistive cells (quasistatic approximation) and used a supercomputer to determine the current distribution that would be induced in the three-dimensional resistive network by

an external magnetic field pulse However, such methods are not generally available and are still an approximation. A means of imaging the induced electric field is what is really needed.

APPENDIX II

Principal Component Analysis And Singular Value Decomposition

Principal Component Analysis

Principal component analysis is a mathematical device that uses the intrinsic spatial and temporal properties of a set of fMRI data to reduce its dimensionality. PCA does not refer to a specific statistical model, entails few assumptions, and makes no comment on the significance of the resulting spatial modes. By orthogonalizing the covariance matrix, PCA extracts its important features in terms of principal components, or eigenvectors. These vectors are the linear combinations that account for independent or orthogonal amounts of variance in the observed data. In terms of functional connectivity, a principal component represents a spatially distributed brain system, comprising a subset of brain region, within which many temporal intercorrelations exist. Because any one principal component is orthogonal to the remaining principal components, these systems are functionally unconnected from each other, even though any single area may be implicated in more than one system. To perform PCA, a mathematical technique called *singular value decomposition* is usually used (70).

Singular Value Decomposition

Given a set of M linear algebraic equations relating a set of N unknowns, x_j, j = 1,2, . . .

$$a_{11}x_1 + a_{12}x_2 + \cdots + a_{1N}x_N = b_1$$
$$a_{21}x_1 + a_{22}x_2 + \cdots + a_{2N}x_N = b_2$$
$$\cdots \qquad\qquad \cdots$$
$$a_{M1}x_1 + a_{M2}x_2 + \cdots + a_{MN}x_N = b_M$$

or, in matrix form,

$$A \cdot x = b$$

where the as and bs are known. If $N = M$, there are as many equations as unknowns, and there is a good chance of finding a unique solution set of x_js.

If $M < N$ or $M = N$ but the equations are not all linearly independent, then there are effectively fewer equations than unknowns. In this case, either there is no solution, or else there is more than one solution vector x. In the latter event, the solution space consists of a particular solution x_p added to any linear combination of (typically) N-M vectors (which are said to be in the nullspace of the matrix A). The task of finding the solution space of A is called *singular value decomposition* of a matrix A.

Singular value decomposition explicitly constructs orthonormal bases for the nullspace (N-M dimensions) and the range (M dimensions) of the matrix, finding the least-squares best compromise solution.

REFERENCES

1. Grafman J. TMS as a primary brain mapping tool. In: George MS, Belmaker RH, eds. *Transcranial magnetic stimulation (TMS) in neuropsychiatry.* Washington, DC: American Psychiatric Press, 2000:115–140.
2. George MS, Belmaker RH. *Transcranial magnetic stimulation in neuropsychiatry.* Washington, DC: American Psychiatric Press, 2000.
3. George MS, Lisanby SH, Sackeim HA. Transcranial magnetic stimulation: applications in neuropsychiatry. *Arch Gen Psychiatry* 1999;56:300–311.
4. Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of the human motor cortex. *Lancet* 1985;1:1106–1107.
5. Saypol JM, Roth BJ, Cohen LG, et al. A theoretical comparison of electric and magnetic stimulation of the brain. *Ann Biomed Eng* 1991;19:317–328.
6. Roth BJ, Saypol JM, Hallett M, et al. A theoretical calculation of the electric field induced in the cortex during magnetic stimulation. *Electroencephalogr Clin Neurophysiol* 1991;81:47–56.
7. Bohning DE. Introduction and overview of TMS physics. In: George MS, Belmaker RH, eds. *Transcranial magnetic stimulation in neuropsychiatry.* Washington, DC: American Psychiatric Press, 2000:13–44.
8. Bohning DE, Pecheny AP, Epstein CM, et al. Mapping transcranial magnetic stimulation (TMS) fields *in vivo* with MRI. *Neuroreport* 1997;8:2535–2538.
9. Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop in the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996. *Electroencephalogr Clin Neurophysiol* 1998;108:1–16.
10. Post RM, Kimbrell TA, Frye M, et al. Implications of kindling and quenching for the possible frequency dependence of rTMS. *CNS Spectrums: the International Journal of Neuropsychiatric Medicine* 1997;2:54–60.
11. Pascual-Leone A, Valls-Sole J, Wasserman EM, et al. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain* 1994;117:847–858.
12. Wassermann EM, Wedegaertner FR, Ziemann U, et al. Crossed reduction of motor cortex excitability by 1 Hz transcranial magnetic stimulation. *Neurosci Lett* 1998;250:141–144.
13. Kimbrell TA, Little JT, Dunn RT, et al. Frequency dependence of antidepressant response to left prefrontal repetitive transcranial magnetic stimulation (rTMS) as a function of baseline cerebral glucose metabolism. *Biol Psychiatry* 1999;46:1603–1613.
14. Lorberbaum JP, Wassermann EM. Safety concerns of transcranial magnetic stimulation. In: George MS, Belmaker RH, eds. *Transcranial magnetic stimulation in neuropsychiatry.* Washington, DC: American Psychiatric Press, 2000:141–162.
15. Epstein CM, Lah JJ, Meador K, et al. Optimum stimulus parameters for lateralized suppression of speech with magnetic brain stimulation. *Neurology* 1996;47:1590–1593.
16. Fox PT, Mintun MA. Noninvasive functional brain mapping by change-distribution analysis of averaged PET images of $H_2{}^{15}O$ tissue activity. *J Nucl Med* 1989;30:141–149.

17. Bandettini PA, Jesmanowicz A, Wong EC, et al. Processing strategies for time-course data sets in functional MRI of the human brain. Magn Reson Med 1993;30:161–173.
18. Strother SC, Kanno I, Rottenberg DA. Principal component analysis, variance partitioning, and "functional connectivity." *J Cereb Blood Flow Metab* 1995;15:353–360.
19. Friston KJ, Frith CD, Liddle PF, et al. Functional connectivity: the principal-component analysis of large (PET) data sets. *J Cereb Blood Flow Metab* 1993;13:5–14.
20. Friston KJ. Functional and effective connectivity in neuroimaging: a synthesis. *Hum Brain Mapping* 1994;2:56–78.
21. McIntosh AR, Gonzalez-Lima F. Structural equation modeling and its application to network analysis in functional brain imaging. *Hum Brain Mapping* 1994;2:2–22.
22. Friston KJ, Worsley KJ, Frackowiak RSJ, et al. Assessing the significance of focal activations using their spatial extent. *Hum Brain Mapping* 1994;1:210–220.
23. Gerstein GL, Perkel DH. Simultaneously recorded trains of action potentials: analysis and functional interpretation. *Science* 1969;164:828–830.
24. Gerstein GL, Bedenbaugh P, Aertsen AM. Neuronal assemblies. *IEEE Trans Biomed Eng* 1989;36:4–14.
25. Friston KJ. Commentary and opinion: II. Statistical parametric mapping: ontology and current issues. *J Cereb Blood Flow Metab* 1995;15:361–370.
26. Adler RJ. *The geometry of random fields.* New York: John Wiley and Sons, 1981.
27. Gochin PM, Miller EK, Gross CG, et al. Functional interactions among neurons in inferior temporal cortex of the awake macaque. *Exp Brain Res* 1991;84:505–516.
28. Gevins A, Zeitlin GM, Yingling CD, et al. EEG patterns during cognitive tasks. I. Methodology and analysis of complex behaviors. *Electroencephalogr Clin Neurophysiol* 1979;47:693–703.
29. Gevins A, Doyle JC, Cuttilo BA, et al. Electrical potentials in human brain during cognition: a new method of reveals dynamic patterns of correlation. *Science* 1981;213:918–922.
30. Gevins A, Bressler SL, Morgan NH, et al. Event-related covariances during a bimanual visuomotor task. I. Methods and analysis of stimulus- and response-locked data. *Electroencephalogr Clin Neurophysiol* 1989;74:58–75.
31. Thatcher RW, Krause PI, Hrybyck M. Cortico-cortical associations and EEG coherence: a two-compartment model. *Electroencephalogr Clin Neurophysiol* 1986;64:123–143.
32. Tucker DM, Roth DL, Bair TB. Functional connections among cortical regions: topography of EEG coherence. *Electroencephalogr Clin Neurophysiol* 1986;63:242–250.
33. Friston KJ, Frith CD, Fletcher P, et al. Functional topography: multidimensional scaling and functional connectivity in the brain. *Cereb Cortex* 1996;6:156–164.
34. Paus T, Jech R, Thompson CJ, et al. Transcranial magnetic stimulation during positron emission tomography: a new method for studying connectivity of the human cerebral cortex. *J Neurosci* 1997;17:3178–3184.
35. Alexander GE, Moeller JR. Application of the scaled subprofile model to functional imaging in neuropsychiatric disorders: a principal component approach to modeling brain function in disease . *Hum Brain Mapping* 1994;2:79–94.
36. McIntosh AR, Grady CL, Ungerleider LG, et al. Network analysis of cortical visual pathways mapped with PET. *J Neurosci* 1994; 14:655–666.
37. Friston KJ, Frith CD, Frackowiak RSJ. Time-dependent changes in effective connectivity measured with PET. *Hum Brain Mapping* 1993;1:69–79.
38. Aertsen AMH, Gerstein GL, Habib MK, et al. Dynamics of neuronal firing correlation: modulation of "effective connectivity." *J Neurophysiol* 1989;61:900–917.
39. Loehlin JC. *Latent variable models: an introduction to factor, path and structural analysis.* Hillside, NJ: Lawrence Erlbaum Associates, 1987.
40. Wassermann EM, Kimbrell TA, George MS, et al. Local and distant changes in cerebral glucose metabolism during repetitive transcranial magnetic stimulation (rTMS). *Neurology* 1997;48: A107-P02.049(abst).
41. Kimbrell TA, George MS, Danielson AL, et al. Changes in cerebral metabolism during transcranial magnetic stimulation. *Biol Psychiatry* 1997;41:108S-#374(abst).
42. George MS, Wassermann EM, Kimbrell T, et al. An overview of initial studies combining conventional functional imaging (PET, SPECT, fMRI) with transcranial magnetic stimulation (TMS) to actively probe brain-behavior relationships. *J Neuropsychiatry Clin Neurol* 1997;9:131.
43. Stallings LE, Speer AM, Spicer KM, et al. Combining SPECT and repetitive transcranial magnetic stimulation (rTMS)—left prefrontal stimulation decreases relative perfusion locally in a dose-dependent manner. *Neuroimage* 1997;5:S521(abst).
44. Chen W, Zhu X-H, Ogawa S, et al. Probing neural events by fMRI at the neural time scale of milliseconds. *Proceedings of the International Society of Magnetic Resonance in Medicine* 2000;8: #501(abst).
45. Cohen LG, Roth BJ, Nilsson J, et al. Effects of coil design on delivery of focal magnetic stimulation. Technical considerations. *Electroencephalogr Clin Neurol* 1990;75:350–357.
46. Wassermann EM, Wang B, Zeffiro TA, et al. Locating the motor cortex on the MRI with transcranial magnetic stimulation and PET. *Neuroimage* 1996;3:1–9.
47. Roberts DR, Vincent DJ, Speer AM, et al. Multi-modality mapping of motor cortex: comparing echoplanar BOLD fMRI and transcranial magnetic stimulation. *J Neural Transm* 1997;104: 833–843.
48. Talairach J, Tournoux P. *Co-planar stereotaxic atlas of the human brain: 3-dimensional proportional system: an approach to cerebral imaging.* New York: Thieme Medical Publishers, 1988.
49. Peters T, Davey B, Munger P, et al. Three-dimensional multimodal image guidance for neurosurgery. *IEEE Trans Med Imaging* 1996;15:121–128.
50. Krings T, Buchbinder BR, Butler WE, et al. Transcranial magnetic stimulation and functional magnetic resonance imaging: complementary approaches in the evaluation of cortical motor function. *Neurology* 1997;48:1406–1416.
51. Bohning DE, Shastri A, Nahas Z, et al. Echoplanar BOLD fMRI of brain activation induced by concurrent transcranial magnetic stimulation (TMS). *Invest Radiol* 1998;33:336–340.
52. Bohning DE, Shastri A, McConnell K, et al. A combined TMS/ fMRI study of intensity-dependent TMS over motor cortex. *Biol Psychiatry* 1999;45:385–394.
53. Shastri A, George MS, Bohning DE. Performance of a system for interleaving transcranial magnetic stimulation with steady state magnetic resonance imaging. *Electroencephalogr Clin Neurophysiol* 1999;51:55–64.
54. George MS, Wassermann EM, Williams WA, et al. Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. *Neuroreport* 1995;6:1853–1856.
55. George MS, Stallings LE, Speer AM, et al. Prefrontal repetitive transcranial magnetic stimulation (rTMS) changes relative perfusion locally and remotely. *Hum Psychopharmacol* 1999;14: 161–170.
56. Josephs O, Athwal BS, Mackinnon C, et al. Transcranial magnetic stimulation with simultaneous undistorted functional magnetic resonance imaging. *Proceedings of the seventh annual meeting of the International Society of Magnetic Resonance in Medicine* 1999:1696.
57. Bohning DE, Shastri A, Wassermann EM, et al. BOLD-fMRI

response to single-pulse transcranial magnetic stimulation (TMS). *J Magn Reson Imaging* 2000;11:569–574.
58. Fox P, Ingham R, George MS, et al. Imaging human intra-cerebral connectivity by PET during TMS. *Neuroreport* 1997;8: 2787–2791.
59. Paus T, Jech R, Thompson CJ, et al. Dose-dependent reduction of cerebral blood flow during rapid-rate transcranial magnetic stimulation of the human sensorimotor cortex. *J Neurophysiol* 1997;79:1102–1107.
60. Nahas Z, Speer AM, Molloy M, et al. Preliminary results concerning the roles of frequency and intensity in the antidepressant effect of daily left prefrontal rTMS. *Biol Psychiatry* 1998;43:94s-#315(abst).
61. Nahas Z, Lomarev M, Roberts DR, et al. Unilateral left prefrontal transcranial magnetic stimulation produces intensity-dependent bilateral effects as measured with interleaved BOLD fMRI. *Biol Psychiatry (in press).*
62. Ilmoniemi RJ, Virtanen J, Ruohonen J, et al. Neuronal response to magnetic stimulation reveals cortical reactivity and connectivity. *Neuroreport* 1997;8:3537–3540.
63. Kosslyn SM, Pascual-Leone A, Felician O, et al. The role of area 17 in visual imagery: convergent evidence from PET and rTMS. *Science* 1999;284:167–170.
64. Desmurget M, Epstein CM, Turner RS, et al. Role of the posterior parietal cortex in updating reaching movements to a visual target. *Nat Neurosci* 1999;2:563–567.
65. Tofts PS. The distribution of induced currents in magnetic stimulation of the nervous system. *Phys Med Biol* 1990;35:1119–1128.
66. Branson NM, Tofts PS. Analysis of the distribution of current induced by a changing magnetic field in a volume conductor. *Phys Med Biol* 1991;36:161–168.
67. Cohen LG, Cuffin BN. Developing a more focal magnetic stimulator. Part I: some basic principles. *J Clin Neurophysiol* 1991;8: 102–111.
68. Roth BJ, Saypol JM, Hallett M. A theoretical calculation of the electric field induced in the cortex during magnetic stimulation. *Electroencephalogr Clin Neurophysiol* 1991;81:47–56.
69. Cerri G, DeLeo R, Moglie F, et al. An accurate 3-D model for magnetic stimulation of the brain cortex. *J Med Eng Technol* 1995;19:7–16.
70. Press WH, Flannery BP, Teukolsky SA, et al. *Numerical recipes in C: the art of scientific computing.* New York: Cambridge University Press, 1986.
71. Bohning DE, Shastri A, McGavin L, et al. Brain activity for transcranial magnetic stimulation (TMS) induced and volitional movement are similar in location and level. *Invest Radiol* 2000; 35:676–683.

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