

# Imaging

## *Window on the Brain*

John C. Mazziotta, MD, PhD

**G**iven the rapid advances in neuroimaging and brain mapping, it is no surprise that a whole host of new and burgeoning techniques are on the horizon as we enter the 21st century. This brief review focuses on methods that are just entering the clinical experience or are now being explored in a research setting but have the imminent potential for clinical use. A number of brain mapping techniques now allow the clinician to monitor disease progression and therapeutic effects in either the routine clinical setting or experimental clinical trials. A battery of methods are now available for the preoperative and intraoperative evaluation of patients with lesions in or near critical cortical areas or for targeting purposes when deep nuclei of the brain are potential sites of therapeutic ablation or electrophysiologic stimulation. The development of probabilistic atlases will soon provide a means of understanding normal variants of human brain structure and function and studying brain disorders and their treatment in an objective and quantifiable fashion. Techniques that are now on the horizon for imaging gene expression, neuronal excitivity, and connectivity are presented in their current stage of development. It is clear that brain mapping and neuroimaging will continue to be ever more important parts of clinical neuroscience and may ultimately serve as the bridge between the molecular and clinical domains of this field.

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Brain mapping methods and neuroimaging techniques have improved and advanced rapidly during the last 100 years. The methods have resulted in improvements in diagnostics and monitoring of therapeutics and have provided insights into the basic mechanisms of cerebral disorders. Never before have so many methods been available to tackle the problems of neurological, neurosurgical, and psychiatric diseases of the brain. In a review article of this length, it is not possible to provide an encyclopedic description of these methods. In fact, many excellent reviews and texts are currently available, and the reader is referred to them for a more in-depth discussion of the general topic of brain mapping.<sup>1-6</sup> In this review, I will focus on emerging or newly established techniques that should fur-

ther augment the methods currently available to evaluate patients with brain disorders. Some of these methods are currently in use, others are being tested only in the research environment, and a few are just beginning to emerge; their potential uses in clinical neuroscience will reflect my own optimistic speculations. These areas include the use of brain mapping techniques to monitor disease progression and therapeutics, their preoperative and intraoperative use in the surgical evaluation of patients, the development of probabilistic atlases to study brain disorders and their use in experimental trials, and, finally, the imaging of gene expression, neuronal excitability, and connectivity. The mere fact that one can contemplate a list of uses for brain mapping methods that includes such a wide range of clinically important measurements is testimony in itself to the progress and future potential of this field.

*From the Departments of Neurology, Radiological Sciences, and Medical and Molecular Pharmacology, Ahmanson-Lovelace Brain Mapping Center, UCLA School of Medicine, Los Angeles, Calif.*

## Advantages and Limitations of Current Brain Mapping Techniques, Their Applications, and Range of Measurements

Method	Measurements Provided	Disorders	Advantages	Limitations
x-Ray computed tomography (CT)	Brain structure, blood-brain barrier integrity	Acute or chronic hemorrhages, acute trauma, general screening of anatomy, focal or generalized atrophy, hydrocephalus	Excellent bone imaging, ≈100% detection of hemorrhages, short study time, can scan patients with ancillary equipment, can scan patients with metal or electronic devices	Ionizing radiation, poor contrast resolution
Magnetic resonance imaging	Brain structure, brain and cervical vasculature, relative cerebral perfusion, chemical concentrations, fiber tracts, blood-brain barrier integrity	Acute ischemia, neoplasms, demyelinating disease, epileptic foci, degenerative disorders, infections, preoperative mapping	High spatial resolution; no ionizing radiation; high gray-white contrast; no bone-generated artifact in posterior fossa; can also perform chemical, functional, and angiographic imaging	Long study duration, patients may be claustrophobic, electronic devices contraindicated, acute hemorrhages problematic, relative measurements only
Positron emission tomography	Perfusion, metabolism, substrate extraction, protein synthesis, neurotransmitter integrity, receptor binding, blood-brain barrier integrity	Ischemic states, degenerative disorders, epilepsy, movement disorders, affective disorders, neoplasms, addictive states, preoperative mapping	Can perform hemodynamic, chemical, and functional imaging; quantifiable results; absolute physiologic variables can be determined; uniform spatial resolution	Ionizing radiation, high initial costs, long development time for new tracers, limited access, low temporal resolution
Single photon emission CT	Perfusion, neurotransmitter integrity, receptor binding, blood-brain barrier integrity	Ischemic states, degenerative disorders, epilepsy, movement disorders	Can perform hemodynamic, chemical, and functional imaging; widely available	Ionizing radiation, relative measurements only, nonuniform spatial resolution, low temporal resolution
Xenon-enhanced CT	Perfusion	Ischemic states	Uses existing equipment	Ionizing radiation, high xenon concentrations have pharmacologic effects
Helical CT angiography	Vascular anatomy, bony anatomy	Vascular occlusive disease, aneurysms, arteriovenous malformations	Provides high-resolution vascular images	Ionizing radiation, vascular and bony anatomy only
Electroencephalography	Electrophysiology	Epilepsy, encephalopathies, degenerative disorders, preoperative mapping	No ionizing radiation, high temporal resolution, widely available, can identify epileptic foci	Low spatial resolution, weighted toward surface measurements
Magnetoencephalography	Electrophysiology	Epilepsy	No ionizing radiation, high temporal resolution, can identify epileptic foci	Low spatial resolution
Transcranial magnetic stimulation	Focal brain activation	Preoperative mapping	No ionizing radiation, potential for therapy, can be linked to other imaging methods (positron emission tomography, functional magnetic resonance imaging)	Low spatial resolution, has produced seizures in certain patient groups
Optical intrinsic signal imaging	Integrated measure of blood volume, metabolism, and cell swelling	Intraoperative mapping	No ionizing radiation, high temporal resolution, high spatial resolution	Complex signal source, invasive only (intraoperative)

### GENERAL ISSUES

It is important to keep in mind that individual brain imaging methods typically address only one aspect, or at best a few aspects, of underlying cerebral physiology and pathophysiology (**Table**). For example, electromagnetic physiologic techniques, such as electroencephalography (EEG), event-related potentials, and magnetoencephalography, provide information about large constellations of neurons and the net electromagnetic physiologic vector that their activity produces. These methods have the best temporal resolution currently available but are lacking in regard to spatial resolution and localization accuracy. Other techniques are devoted specifically to measurements of brain structure.

These include x-ray computed tomography (CT), conventional magnetic resonance imaging (MRI), and blood vessel imaging using conventional angiography, magnetic resonance angiography, or helical CT.<sup>7</sup> A third group of methods assesses hemodynamic response as a measure of brain function. Techniques in this group include xenon-enhanced CT, functional MRI (fMRI), perfusion MRI (pMRI), and cerebral blood flow or volume measurements obtained with either positron emission tomography (PET) or single photon emission computed tomography (SPECT). These methods assume that neuronal firing and blood flow increments or decrements are tightly coupled. This seems reasonable in the normal brain, but it may not always be true in pathologic states. Methods that determine the chemical processes

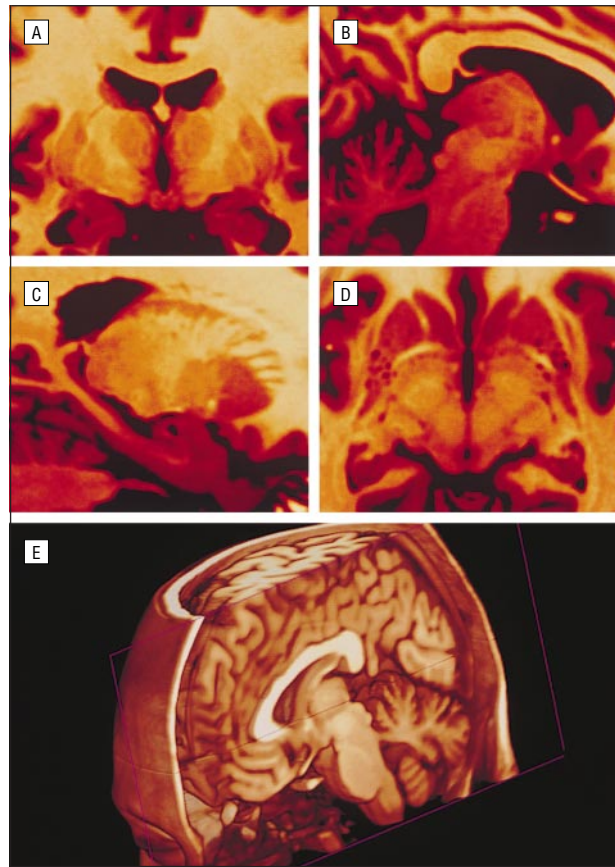
of the brain fall mainly in the domain of PET and magnetic resonance spectroscopy. PET is able to measure glucose metabolism, protein synthesis, amino acid uptake, pH, and other variables in a quantitative manner with results calculated in the appropriate physiologic units. Magnetic resonance spectroscopy provides relative measurements of chemical compounds typically derived from hydrogen proton spectra, but, at higher magnetic field strengths, it is also possible to estimate relative quantities of molecules containing resonant isotopes of sodium, fluorine, carbon, and phosphorus. Imaging of brain receptor systems, both neurotransmitter molecules and receptor complexes, has been an active area of research in both health and disease using PET and SPECT. Most information has been derived for the dopaminergic system, but data also exist for the cholinergic, serotonergic, opioid, and benzodiazepine systems.

Also, there are interactive approaches. The most time-honored, of course, is the direct observation of signs and symptoms in patients with cerebral disorders. This is always the necessary prelude for any imaging evaluation of a patient. An invasive, but still frequently used approach, is the circumstance of awakening patients from anesthesia during surgical procedures and recording or stimulating cerebral tissue, correlating the results with behavioral states in a conscious patient. Recently, such measurements have been augmented through the use of optical intrinsic signal (OIS) imaging in which changes in optical reflectance from the cortex are measured intraoperatively during stimulation.<sup>8</sup> Measurements can be made either in awake patients during the performance of tasks or in anesthetized patients receiving sensory stimulation. Optical intrinsic signal provides measures of function for different brain regions in the operative field.

Finally, the technique of transcranial magnetic stimulation (TMS) allows the investigator to stimulate the cortex of the brain magnetically through the skull, resulting in an induced electrical discharge in the cortex. By observing the behavior of the subject during such stimulation, maps of function can be produced.<sup>9,10</sup>

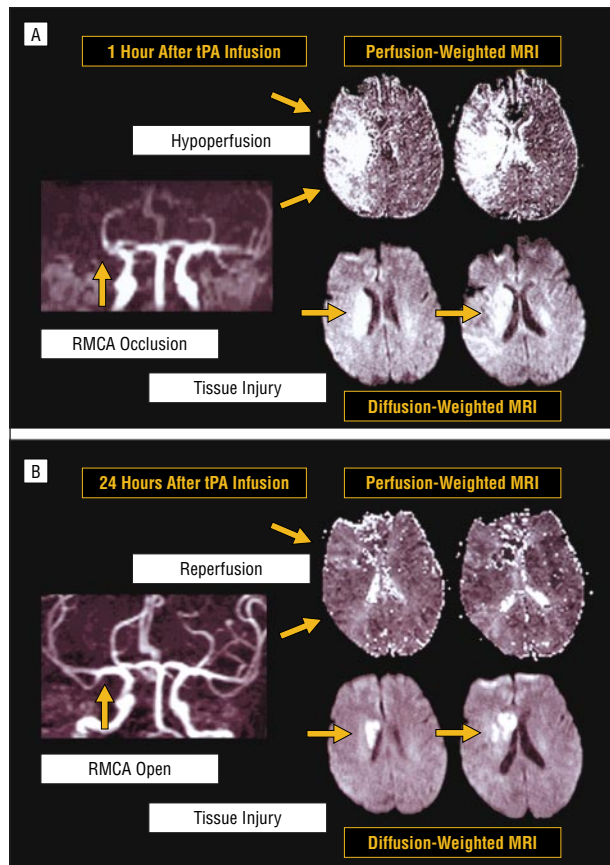
#### MONITORING DISEASE PROGRESSION, PROGNOSIS, AND THERAPY

There are a host of techniques currently available for diagnostic, prognostic, and therapeutic monitoring purposes. The most commonly used of all these techniques is currently MRI<sup>11</sup> (**Figure 1**). The spatial and contrast resolution of this method surpasses all others, and it is the structural imaging study of choice in all situations except when contraindicated by implanted ferromagnetic devices, claustrophobia, or when patients are too unstable to make a relatively long examination in the magnet safe. An excellent example of how MRI is transforming clinical practice can be illustrated by the evaluation of patients with ischemic cerebrovascular disease. Because of the urgency associated with current thrombolytic approaches to ischemic brain injury, rapid and complete evaluation of a patient with MRI has emerged as a key prelude to therapy. In the appropriate patient, less than 3 hours from the acute onset of symptoms, a detailed image of structural brain anatomy and the presence of cerebral hemorrhage or preexisting infarction can rapidly be determined. At the same time, vascular



**Figure 1.** A through D, Two-dimensional magnetic resonance images of the brain obtained by serially imaging, averaging, and reconstructing a final image. Although performed on a conventional 1.5-T scanner, this averaging strategy preserves signal and reduces noise, resulting in images that have extraordinary anatomic detail.<sup>12</sup> A, Coronal view through the thalamus demonstrating its subnuclei; the mammillary bodies; the internal, external, and extreme capsules; and the 2 segments of the globus pallidus. Also note the detailed anatomy of the hippocampi. B, Sagittal view demonstrating the colliculi of the midbrain, the nuclei of the thalamus, and the midsagittal region of the cerebellum. C, Sagittal view through the hippocampus and striatum. Note the fine bridges of gray matter between the caudate and the putamen. D, Transverse section through the basal ganglia and upper midbrain. Note the periaqueductal gray matter, the detailed anatomy of the hippocampi, and the bilateral flow voids produced by the presence of the lenticulostriate arteries in the posterior portion of the putamen. E, Three-dimensional reconstruction with cutaway of a magnetic resonance imaging data set demonstrating the kind of anatomic detail that can be provided with 3-dimensional magnetic resonance images. These figures were provided by Colin Holmes, PhD, and colleagues, UCLA School of Medicine, Los Angeles, Calif.

anatomy is assessed with magnetic resonance angiography (**Figure 2**). Early tissue injury can next be identified by obtaining diffusion-weighted images that demonstrate changes in intracellular and extracellular spaces associated with early ischemia by virtue of alterations in the normal pattern of water (proton) diffusion in the brain.<sup>13-15</sup> By combining these data with measurements of relative cerebral perfusion (pMRI), obtained by bolus administration of gadolinium-labeled intravascular compounds, the location, extent, temporal course, vascular anatomy, and volume of jeopardized (hypoperfused), ischemic (altered diffusion), or infarcted tissue can be assessed. The results provide an estimate of the patient's prognosis and a direction for therapeutic intervention, which can then be monitored using the same methods (**Figure 2**).



**Figure 2.** Magnetic resonance imaging (MRI) study of a patient with ischemic cerebral vascular disease before and after intra-arterial thrombolytic therapy. A, Acute occlusion of the right middle cerebral artery (RMCA) demonstrated on magnetic resonance angiography. Note that in the perfusion-weighted MRI, there is a large area of hypoperfusion (seen as white), encompassing the entire RMCA distribution and extending all the way from the cortical surface to the lateral ventricle. In addition, the diffusion-weighted MRI demonstrates tissue injury and cell swelling in the central portion of this MCA distribution closest to the ventricle (arrows, white region). B, Twenty-four hours following thrombolytic therapy (tissue-type plasminogen activator [tPA] infusion), magnetic resonance angiography demonstrated patency of the RMCA and resolution of the perfusion deficit on the perfusion-weighted MRI study. There is a residual area of tissue injury (the white area on the diffusion-weighted MRI), but this residual tissue damage represents only a small portion of the large volume of tissue at risk when compared with the study shown in the top section. Such an approach can be used to select patients for thrombolytic therapy, monitor their outcome, and determine the clinical effectiveness of such an approach. These figures were provided by Steven Warach, MD, PhD, National Institute of Neurological Disorders and Stroke, Bethesda, Md, and colleagues at the Beth Israel Deaconess Medical Center, Boston, Mass.

Currently, MRI does not allow for the measurement of oxygen extraction or metabolism, but magnetic resonance spectroscopy has been used to evaluate lactate concentrations in ischemic tissue. Perfusion, metabolism, and oxygen or glucose extraction can also be quantified with PET, although few sites have such methods available for studies on an urgent basis. Functional imaging with fMRI, PET, or SPECT can be used to evaluate disease progression and either experimental or clinical therapy in patients with neurodegenerative disorders such as Alzheimer, Parkinson, and Huntington disease.<sup>6</sup> Measurements of baseline metabolism or increments in cerebral perfusion during behavioral tasks can be used as a surrogate measure of therapeutic success or failure. This is of particular value in presymptomatic patients (eg, patients with genetically proven expanded tri-

nucleotide repeats who are not yet symptomatic from Huntington disease), since there is no clinical measure to monitor in assessing the efficacy of interventions in such a situation.

A particularly important area for using functional imaging methods is in the evaluation of patients before, during, and after neurorehabilitation with behavioral, pharmacologic, or combinations of interventions. Since these imaging methods allow the observation of plastic changes in the functional organization of the brain, they may be used to identify individuals who might benefit most from such interventions as well as which interventions are best suited for a given clinical situation. Further, the timing of the interventions may be assessed by longitudinal evaluation of experimental subjects when compared with control populations for each type of neurorehabilitatory approach. Given the fact that there are an estimated 54 million neurologically disabled individuals in the United States, methods that optimize both the type and timing of neurorehabilitation interventions should prove extremely important and cost-effective in the future evaluation of such individuals.

## SURGICAL PLANNING

The use of brain mapping methods to target sites in the brains of patients with cerebral disorders who are surgical candidates has 2 potential goals. The first is to identify sites in the brain for ablation therapy because they themselves are the cause of a pathologic condition, such as neoplasms or vascular malformations. The second is when surgery is planned to interrupt specific pathways that are contributing to a patient's symptoms, such as in Parkinson disease. In either case, optimal anatomic detail and, ideally, functional characterization should improve the operative results.<sup>6</sup>

Modern brain mapping techniques are helpful in the targeting process in 4 ways. First, improved lesion localization and characterization can help better identify sites for ablation. Second, increased anatomic resolution with structural imaging techniques provides for better anatomic definition of landmarks at or near the target. Third, functional imaging may allow for the physiologic activation of targeted sites, particularly in those situations where normal circuits are intended to be interrupted for a therapeutic purpose, as in Parkinson disease. Fourth, the use of population-based, probabilistic atlases<sup>16</sup> that account for variance in structure and function between subjects can improve the identification of structural and functional sites of importance in a given subject's brain.

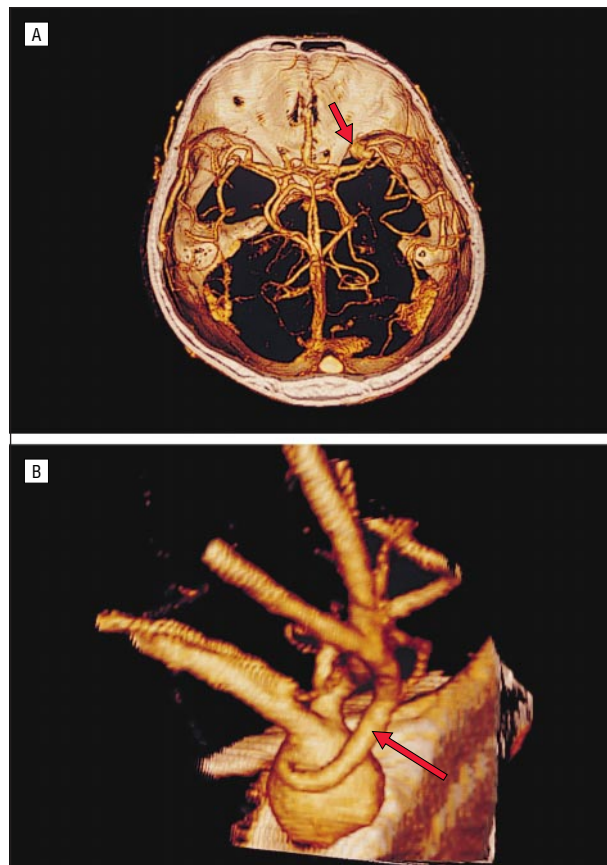
There is little doubt that the advances in MR imaging have produced anatomic data sets that are beyond any previous level of detail, both in terms of spatial resolution and contrast (Figure 1). In addition, high-speed MR imaging units allow the approach of averaging multiple studies, enhancing spatial resolution, and allowing the visualization of structures previously unseen with *in vivo* imaging, all of which are important in targeting strategies (Figure 1).<sup>12</sup>

Less progress has been made in the functional activation of sites that are potential sources of either stimulation or ablation (eg, globus pallidus, thalamic nuclei). Significant advances in both the paradigms and the imaging strategies will be required before reliable targeting of such functional sites can be used as a preoperative method. It is also possible that selective ligand studies can identify specific

neurotransmitter systems of interest for either ablation or stimulation as a means of therapeutic intervention.

The development of helical CT is the latest advance in the ability to perform imaging of the vascular system of the brain and neck in a fashion that is of particular interest for presurgical planning.<sup>7</sup> Here, high-speed CT imaging during bolus administration of iodinated intravenous contrast is coupled with the movement of the patient bed through the scanner gantry. In one vascular transit time, the entire head is imaged. This strategy has been used to provide high-resolution images of the 3-dimensional structure of the normal vascular anatomy of the brain and aneurysms, arteriovenous malformations, and other vascular abnormalities (**Figure 3**). Conventional angiography results in images that are the 2-dimensional projections of the blood vessel lumens. Often the neck of an aneurysm and its relation to its parent or daughter arteries can be hard to discern unless a number of different views are obtained at different angles. This exposes the patient to multiple doses of radiation and an increased burden of iodinated contrast material. Using helical CT, a single bolus contrast administration allows for the reconstruction of the entire vascular complex of the aneurysm as well as its parent and daughter vessels. The subsequent 3-dimensional reconstruction can be rotated and displayed from any angle, allowing the neurovascular surgical team to closely examine these relations and plan a preoperative surgical strategy. This improved information occurs without the requirement for multiple radiation or contrast exposures to the patient. In addition, it may lead the way to the development of virtual clips or other surgical instruments uniquely designed for each patient's lesion that can be tested in a virtual reality environment and then manufactured before the actual procedure.

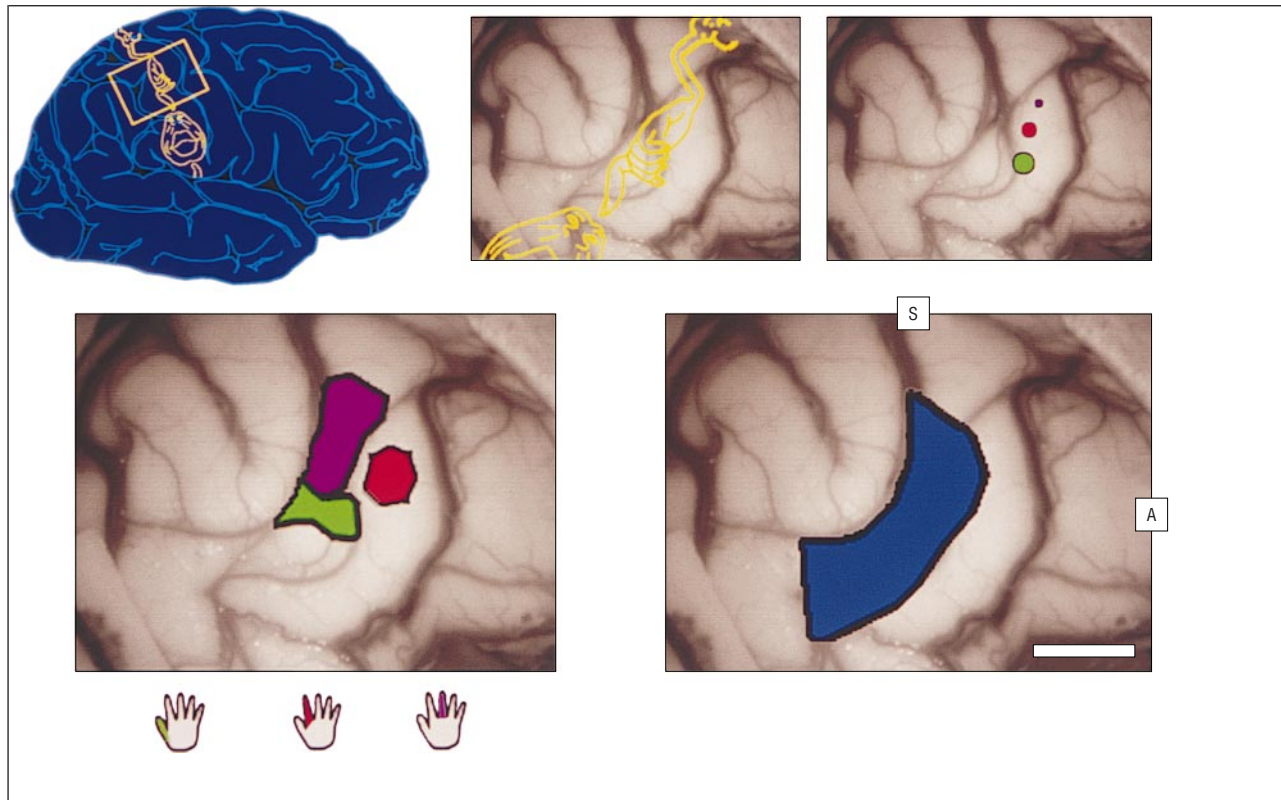
Patients with lesions of the cerebral hemispheres that require surgical resection or neuroradiologic intervention run the risk of losing normal functions controlled by adjacent tissues, particularly in the cortex. Such procedures are particularly worrisome in patients with lesions in or near motor or language cortices. Such patients, in the past, have been advised either not to have surgery or to anticipate a significant deficit. In other situations, patients have had their anesthesia reversed once the cortex is exposed, and behavioral testing is conducted while sites of potential cortical resection are stimulated electrically to temporarily disrupt their functional activity. Such measures have been warranted because of the devastating consequences of injury to or removal of cortex critical to movement or language function. In patients who have been candidates for resections of the temporal lobe, testing of memory function has also been part of their presurgical evaluation. In this situation, barbiturates are injected intra-arterially, temporarily, and reversibly, inactivating the mesiotemporal lobe or portions of the entire hemisphere, depending on which vessel is injected. During the pharmacologic window of the drug, patients are behaviorally tested to determine whether they can learn new information. The assumption is that if the temporal lobe that is slated for removal is temporarily anesthetized by the barbiturate administration, the remaining portions of the brain should be capable of memory function and the acquisition of new material for learning purposes. Thus, the Wada procedure, as it is called, is used to determine whether these functions will be retained after the sur-



**Figure 3.** Helical computed tomographic (CT) angiography. A, The cerebral vasculature is superimposed on this cutaway view of the skull seen from above. Note the fine detail of the vascular structures, including the circle of Willis, the anterior, middle, and posterior cerebral arteries, and their branches. The red arrow indicates an aneurysm arising from the middle cerebral artery at the anterior edge of the middle cerebral fossa. B, Close-up view of the aneurysm demonstrated in part A. Note that in this 3-dimensional reconstruction it is possible to see all the blood vessels that contribute to or arise from the aneurysmal sac (seen on the left). Unlike conventional angiography, where the overlap of the aneurysm and parent and daughter vessels can be obscured because of the 2-dimensional projection required, full 3-dimensional images are possible with helical CT angiography. By digital reconstruction and rotation of the data set from helical CT, these complex and important relations can be evaluated before surgery, whereas with conventional angiograms, multiple views would be required and may still not provide a sufficiently detailed view of the relations. In addition, multiple views obtained with conventional angiography expose the patient to additive radiation and contrast risks. In this case, the angular artery (red arrow) arises from the aneurysm sac. Endovascular coil placement might lead to obstruction of this important vessel, thereby making such a patient a candidate for surgical rather than endovascular treatment of this lesion. These figures were provided by Pablo Villablanca, MD, PhD, UCLA School of Medicine, Los Angeles, Calif.

gical resection. Analogous approaches can be used for language areas. These studies are, in fact, difficult to perform and require a good rapport with the patient who needs to be cooperative and motivated to provide accurate data. Barbiturate administrations can be accompanied by transient hemiparesis that, despite pretest warnings, is anxiety producing and adds to the stress and uncertainty of the results.

For all these reasons, noninvasive preoperative mapping has been avidly pursued as the means of trying to avoid some of these more traumatic and potentially less accurate approaches to locating functionally specific, normal cortical areas. PET, SPECT, fMRI, and TMS have all been used for this purpose.<sup>9,17,18</sup> Most data have been collected with



**Figure 4.** Optical intrinsic signal (OIS) imaging. The orientation of individual-digit OIS responses is consistent with the classic homunculus schematic (upper left brain schematic). The middle upper panel applies this schematic over the subject's neuroanatomy. The upper right panel illustrates data from previous studies using single-digit electrocorticography. Distances between median nerve somatosensory evoked potential (SSEP) and individual-digit dipoles were 9, 6, and 5 mm for thumb (green), index finger (red), and middle finger (purple), respectively. When peak OIS responses are displayed over somatosensory cortex, a superior-to-inferior orientation is revealed for the middle finger (purple), index finger (red), and thumb (green), respectively (lower left panel). The distances from the median nerve SSEP phase reversal (within the central sulcus) and maximal sensory OIS responses are 10.7, 4.0, and 5.8 mm for thumb, index finger, and middle finger, respectively. Diameter of digit dipoles indicates  $\pm 2$  SEs of the mean localization. Scale bar is 1 cm. Spatial resolution is 335  $\mu$ m per pixel. A indicates anterior; S, superior. These figures were provided by Arthur Toga, PhD, and colleagues, UCLA School of Medicine, Los Angeles, Calif.

PET and fMRI.<sup>19</sup> In these circumstances, batteries of behavioral paradigms are delivered to patients preoperatively to develop maps of relatively increased perfusion associated with the performance of critical tasks thought to involve brain regions near areas of potential surgical resection. Thus, patients with lesions in or near their sensory-motor cortices are asked to perform tasks that activate the appropriate topographic regions of the motor cortex; the relative distance between the lesion and the activation site provides a measure of the risk involved in the resection. By combining these noninvasive mapping techniques with the conventional Wada test, intraoperative stimulation, and other forms of evaluation, it will be possible to validate the accuracy of the noninvasive approach and determine its applicability for sole use in presurgical patient populations.

Two new tools are available for intraoperative mapping, intraoperative MRI and OIS. Intraoperative MRI can be used with 1 of 2 strategies. In the first, a low-field magnetic resonance unit is installed in the operating room, and the surgeons perform the procedure in the fringe field, moving the patient into the magnet repeatedly to image the progress of the operation. The second approach is to operate within the magnet itself, requiring magnetic resonance-compatible surgical instruments and physiologic monitoring devices. At present, functional imaging with intraoperative MRI has not yet been established.

The newest brain mapping technique to be used intraoperatively is OIS.<sup>8</sup> Using this approach, white light is shown onto the exposed cortex intraoperatively, and the amount of light, at different wavelengths, reflected from the cortex is measured. The reflectance at specific wavelengths of the light changes as a function of the neuronal activity of the illuminated tissue secondary to changes in blood volume and flow, cell swelling, and the oxidative state of the tissue (among other variables). An invasive technique, OIS is used in the operating room to provide functional maps in which neuronal activity is varied by stimulation of peripheral nerves or, in the awake patient, by the performance of behavioral tasks. Notably, OIS has the best spatial and temporal resolution of all the functional imaging techniques, approaching 50  $\mu$ m in the spatial and 50 milliseconds in the temporal domains.<sup>20</sup> Also, OIS can be used to validate preoperative maps from tomographic methods or to continuously identify landmarks identified preoperatively as the brain changes shape, resulting from osmotic dehydration, ventricular drainage, or local edema during the procedure (**Figure 4**).

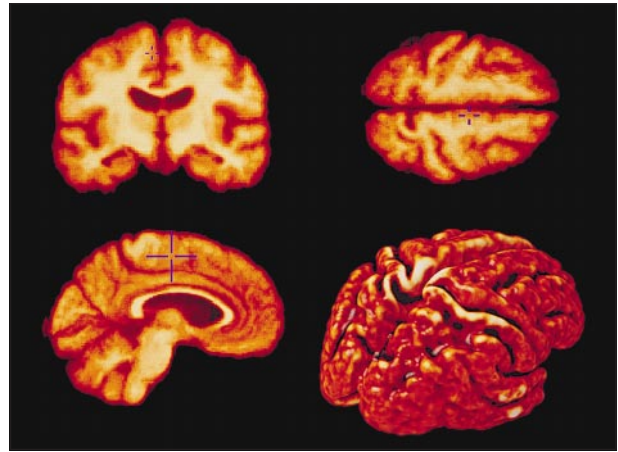
#### PROBABILISTIC ATLASES

The advent of modern mathematical and computational approaches to averaging imaging data across subjects has led to a generation of population-based, probabilistic atlases.<sup>16</sup>

Such atlases are already in existence for the normal brain at different ages. Disease-based atlases may be useful for the differential diagnosis of human cerebral disorders (**Figure 5**).<sup>21</sup>

The basic approach to generating such atlases is to obtain images from a large number of subjects (eg, typically hundreds or thousands) in a mathematical framework that produces a database that is probabilistic. Such an atlas allows the user to obtain relative information that takes into account the variance in structure and function of the human population. Once established, such an atlas can interact with new data sets derived from subjects or patients, both individually and as groups. Thus, a clinician or investigator who performs an MRI scan of a single patient with focal epilepsy could call on a digital probabilistic atlas of healthy subjects and compare the patient's brain with the average normal brain. The variance information, estimated from the population of healthy subjects, is used to determine whether a patient's imaging study falls within or outside normal limits. If the atlas is constructed from a sufficient number of subjects, a subpopulation could be selected that more closely resembles the patient's clinical and demographic profile. In such a case, one might ask for only subjects who are right-handed, female, of a particular race, and between the ages of 25 and 30 years. An increasing number of variables can be included in such a prior specification, depending on the size of the data set constituting the atlas and the range of information collected about the contributing subjects. As a result, it is possible to detect subtle abnormalities of diagnostic importance that would not be identified by the less sensitive conventional approach of qualitatively examining 2-dimensional images. In addition, such an atlas-based approach will give an objective and quantifiable magnitude to any detected abnormality. The scan data from any patient can then be added to the atlas, thereby increasing its value with regard to specific patient groups.

Disease-based atlases are constructed in a similar fashion. It is possible to imagine morphometric and functional atlas for Alzheimer (Figure 5) and Parkinson diseases, schizophrenia, and other disorders.<sup>4,21</sup> Such atlases would provide a population- and disease-based opportunity to examine the natural history of morphometric or functional abnormalities as a function of disease progression, age of onset, genotype, or other variables. Such atlases could be used to identify changes in the natural disease courses that result from therapeutic interventions. Consider, for example, a clinical trial for a new drug for Alzheimer disease. The Alzheimer disease population atlas would provide estimates of morphometric changes (eg, local atrophy) and, for example, alterations in cerebral glucose metabolism as a function of disease progression. Serial imaging of treatment and control groups with the appropriate techniques would then provide 4-dimensional, longitudinal imaging data. Comparisons of morphometric and metabolic changes as a function of time would be undertaken to detect objective and quantitative differences between the 2 groups. Any differences would represent a measure of the effect of therapeutic intervention on progressive atrophy or metabolic change due to the natural history of the disease. It is probable, although currently unproven, that such an approach would be more sensitive in detecting differences between control and experimental groups, providing a surrogate marker of disease



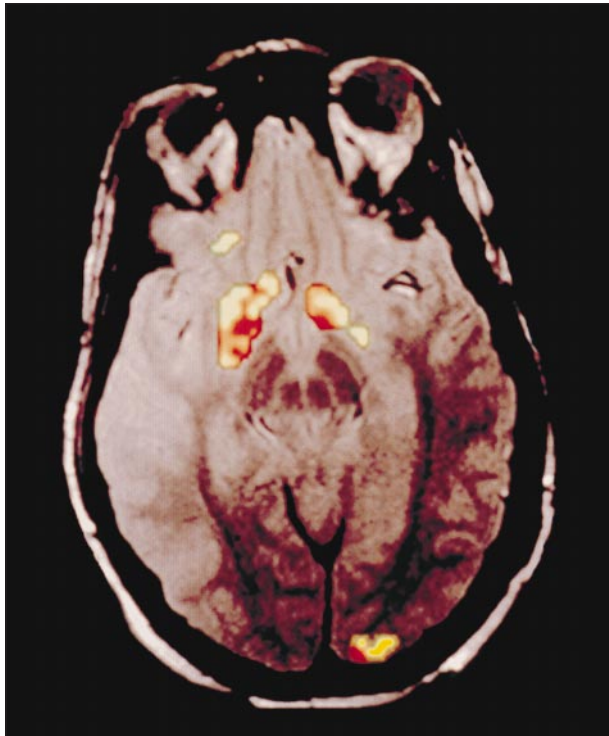
**Figure 5.** Probabilistic population atlas of individuals with Alzheimer disease. This atlas is composed of 9 patients and presented as a set of 2-dimensional orthogonal views plus a 3-dimensional rendering (bottom right), produced using a continuum-mechanical mathematical approach. Note the presence of atrophy on the 3-dimensional image, manifested by the widening of the major fissures of the brain and the sulci in the neocortex. This finding is particularly prominent in the superior parietal lobule. One can also identify thinning of the corpus callosum on the midsagittal view. Such disease-based atlases will be useful not only in tracking the natural history of cerebral disorders but also in providing objective and quantifiable information about structure and function associated with experimental therapies for cerebral disorders. These figures were provided by Paul Thompson, PhD, and colleagues, UCLA School of Medicine, Los Angeles, Calif.

progression and therapeutic effect and, thereby, requiring fewer subjects or shorter time frames for therapeutic assessment, ultimately resulting in lower costs for clinical trials.

## GENE EXPRESSION

The ability to image gene expression in human subjects would provide an important link between the disciplines of molecular biology and phenotype assessment in human patients. These 2 rapidly expanding fields would then be joined with a common purpose, namely, the visualization of molecular events that cause disease and interventions, at a molecular level, that can prevent, delay, arrest, or reverse disease progression. All current approaches require tracer techniques. These can use radioisotope tracers and techniques such as PET and SPECT or nonradioactive contrast agents visible with MRI. The more general the solution to the problem of tracing such events, the more generalizable and useful the result. The approach is similar to the standard molecular biology experiment, where a reporter gene and a reporter probe produce a signal that can be visualized in the cell, typically by some type of optical change (eg, fluorescence). Two promising approaches have been described.

To illustrate the strategy that can be used with PET, consider the situation in which the protein product of the expressed PET reporter gene is an enzyme, such as herpes simplex virus thymidine kinase, and the PET reporter probe is a fluorine 18-labeled analog of ganciclovir, <sup>18</sup>F-fluoroganciclovir.<sup>22</sup> The strategy works as follows. The PET reporter gene is incorporated into the genome of an adenovirus that is administered to the test subject or animal. Subsequently, the <sup>18</sup>F-fluoroganciclovir is injected intravenously. It diffuses into cells, and if there has been no gene expression of the reporter gene, it will dif-



**Figure 6.** Electroencephalographically (EEG) triggered functional magnetic resonance imaging study of a 35-year-old woman with partial epilepsy who had documented epileptic discharges of the temporal lobes with left-sided onset apparent on EEG. Functional magnetic resonance imaging acquisitions, of 2 to 3 seconds each, following the onset of epileptic EEG discharges were compared with baseline, where no discharges were detected. By reconstructing images that occurred during spikes compared with times when no spikes occurred, a resultant “ictal” or “spike map” was produced. Note that the increased signal (colored areas) in the anterior and medial temporal lobes is seen bilaterally associated with the EEG events. This figure was provided by Steven Warach, MD, PhD, John Ives, PhD, and Don Schomer, MD, National Institute of Neurological Disorders and Stroke, Bethesda, Md, and the Beth-Israel Deaconess Medical Center, Boston, Mass.

fuse out of the cells and be cleared from the circulation by the kidneys. If gene expression has occurred, the  $^{18}\text{F}$ -fluoroganciclovir will be phosphorylated by the reporter gene enzyme and trapped in cells. One molecule of the enzyme can phosphorylate many molecules of the reporter probe so that there is an amplification effect in this enzyme-mediated approach.<sup>22</sup>

Two MRI strategies are under consideration for demonstrating gene expression. In the first, a molecule is created with a gadolinium ion at its center, positioned in such a way that the ion has limited access to water protons and, as such, minimally perturbs the MRI signal. Site-specific placement of a portion of the molecule that can be cleaved by the product of a reporter gene then exposes the gadolinium ion, making it more accessible to water protons and changing the magnetic resonance signal at local sites of gene expression. For example, the site-specific placement of a galactopyranosyl ring in the macromolecule can be cleaved by the commonly used reporter gene  $\beta$ -galactosidase.<sup>23</sup> An alternate approach is to create particles composed of DNA and a polylysine molecule modified with a paramagnetic contrast agent such as gadolinium.<sup>23</sup> The cotransport of the DNA and MRI contrast agents demonstrates areas of gene expression that can target specific cells in vivo as a marker of this process.<sup>24</sup> These tools will be extremely useful in

both research and clinical settings as gene therapy becomes a reality in the years to come.

## IMAGING NEURONAL EVENTS AND CONNECTIVITY

Neuronal events take place in the millisecond time frame. Most functional imaging strategies rely on changes in cerebral perfusion or blood volume that typically take seconds to develop. Combine this relatively long physiologic “distance” from the event of importance (ie, one wants to measure neuronal firing but is really measuring perfusion changes) with the methodologic requirements to acquire enough data about events to produce a statistically reliable image and one finds that current functional imaging approaches are integrated events during time frames that are 1 or 2 orders of magnitude longer than the event of interest. Nevertheless, one can use signal averaging techniques to improve on this situation. The EEG recordings obtained in MR imaging devices capable of producing fMRI data have been used to identify the site of epileptic spikes. By continuously scanning subjects as EEG data are recorded, fMRI can be reconstructed at times when spikes occur, adjusting for the appropriate hemodynamic delay<sup>25,26</sup> (**Figure 6**). The same approach can be used for focal seizures provided that the seizures do not produce head movements that would create artifacts in the fMRI data. Once the fMRI data are collected and adjusted for hemodynamic delay, images of relative hyperperfusion are produced associated with the site of interictal spikes or focal seizures (Figure 6). Although the value of such studies in the preoperative evaluation of patients with epilepsy remains to be determined, one of the most difficult problems with such patients is the rapid propagation of seizures from the primary focus to adjacent or remote sites. It is conceivable that such fMRI-EEG data collections may help with this problem. In addition, the same strategy may be used in healthy subjects who repeatedly perform a task, whereas EEG data are collected in the standard event-related potential strategy. With such an approach, the electrophysiologic data from the EEG could be combined with functional data from fMRI, and a composite map having both high spatial and temporal resolutions would be achieved.

An alternate strategy is to look for changes in ionic concentrations that are associated with neuronal events. Here again, 2 strategies can be used. The first is to use MRI and specific natural elements, such as sodium (eg, intracellular vs extracellular), which can be imaged directly in high-field scanners. The second approach is to use molecules that have a “caged” structure and that open at specific ionic concentrations to expose a portion of the molecule that changes the magnetic resonance signal.<sup>27</sup> Caged gadolinium molecules have been developed that open at specific calcium concentrations, potentially giving a local map of calcium concentration in the brain. Similar strategies can be used with different ionic probes. By signal averaging to improve temporal resolution, an image that more closely estimates neuronal activity could be developed. As yet none of these “ionic imaging” strategies have been used in humans.

Finally, one may use a combination of imaging techniques to look at neuronal connectivity.<sup>28</sup> Three techniques are combined. First, consider a healthy subject performing a motor task during an fMRI study to iden-

tify the hand area of the motor cortex. Three-dimensional images are reconstructed, demonstrating the location of that functional area in the subject's brain. The subject is then positioned in a PET device, and a TMS stimulator is targeted to the coordinates of the subject's hand area of the motor cortex. At the time of injection of the PET blood flow tracer, the TMS device is fired, artificially activating the motor cortex, thereby causing it to appear in the functional image. Since this activation will also be propagated orthodromically and antidromically, both inputs and outputs from that subject's motor cortex will also increase in relative perfusion and be visible in the PET image. The strategy allows for the identification of regions of the brain that are functionally connected through the combination of 3 methods. By performing such studies in large numbers of subjects and at many brain locations, such data can be added to probabilistic atlases, discussed herein, to add the important attribute of connectivity to brain structure and function. These can be used in conjunction with anatomic connection data derived using diffusion MRI techniques.<sup>29</sup>

### CONCLUSIONS

Neuroimaging techniques and brain mapping methods clearly have transformed the way we practice clinical neurology and neurosurgery today. The years to come will only see further improvements and opportunities. As the large array of new therapeutic agents enters the field of clinical neuroscience, imaging will provide the means to visualize the success or failure of these strategies and will do so in a quantitative and probabilistic fashion, using modern, objective, and automated strategies that will increase the speed, efficiency, and cost savings of clinical trials in the transition of potential therapies from the bench to the bedside. Ever-improving functional techniques will demonstrate the brain's chemical subsystems, integrated networks, and the complex temporal choreography that result in complex human behaviors, such as learning,<sup>30</sup> and their aberration in disease states. Using some of the methods described herein and others, not yet discovered, imaging will provide the bridges among the phenotype, the genotype, and the behaviors of the human species. By monitoring gene expression, gene therapy, and other molecular events, the continuum between molecular biology and clinical neuroscience will become linked through imaging, allowing us to understand how genetic and cellular events operate in the complex and integrated networks of the human brain.

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Corresponding author: John C. Mazziotta, MD, PhD, Ahmanson-Lovelace Brain Mapping Center, UCLA School of Medicine, 660 Charles E Young Dr, South Los Angeles, CA 90095-7085.

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