Rapid advances and developments in optical imaging technology during the past 15 years have resulted in promising innovations in the realm of high-resolution imaging of the functional architecture of the brain, which has not only contributed to the elucidation of brain functional architecture and plasticity (see reviews1-7) but has also increased our understanding of the temporal and spatial dynamics of cortical seizure spread,1,2,6,8 led to related technical improvements in neurosurgical procedures for the surgical resection of seizure foci,8 and may help to provide more accurate resection of brain tumors9 as well as contribute to our understanding of higher cognitive function.

At present, various methodologies exist for the investigation of brain structure and function, ranging from single-cell electrode recordings to imaging the activity of large populations of neurons. However, they vary widely in their degree of spatial and temporal resolution (Figure 1). Despite being a somewhat invasive technique, optical imaging of intrinsic signals has the advantage of excellent spatial and temporal resolution down to approximately 50 to 100 µm and 150 to 200 milliseconds, respectively, compared with that of functional magnetic resonance imaging at a few millimeters and 1 to 2 seconds (most commonly). The temporal resolution of optical imaging can be improved to a few milliseconds with the use of voltage-sensitive dyes1,2 but such dyes are generally toxic to the brain, precluding their use in human in vivo studies at present. Such high spatial resolution allows the visualization of some of the basic building blocks of the brain’s anatomical and functional organization, eg, the ocular dominance (OD) and orientation columns of visual cortex (Table and Figure 2).

Optical imaging can now resolve this functional architecture as well as other dynamic properties of the cortex in vivo. By simply shining light on the surface of the brain and by recording and analyzing the reflected light patterns, one can obtain high-resolution maps of the functional architecture of the brain on the basis of intrinsic signals (Figure 2).1-3,5,12 Intrinsically signals are generated as a consequence of the metabolic aftereffects of neuronal electrical activity, and therefore indirectly reflect neuronal activity. These signals evolve after a short delay in response to evoked neuronal activity. The source of intrinsic signals has been attributed to changes in microvascular blood volume and flow, changes in light absorption or fluorescence of intrinsic chromophores (eg, hemoglobin), and light-scattering signals from water and ion movement, etc (see reviews1,2,12). In the visible light range (540-630 nm), the signal seems to come primarily from an initial increase in deoxyhemoglobin (starting about 200 milliseconds after the stimulus), followed by an increase in blood volume/flow. A smaller component of the signal is reflected by a rise in oxyhemoglobin level, starting about 1.5 seconds after the stimulus onset.1,2,13 In the near-infrared range, the predominant signal is more from light scattering.1,2,12 Optical imaging may also be performed using voltage-sensitive dyes that are applied to the brain or to brain-slice preparations before re-
cording. The dye binds to electrically excitable membranes and transforms membrane potentials into optical signals that are captured by a photodiode array directly reflecting neuronal activity, hence the better temporal resolution.1,2

HISTORY

The history of optical imaging encompasses the theoretical and practical contributions and observations of many investigators across several decades. It had long been known that changes in the optical properties of nervous tissue occurred with electrical or metabolic activity, but these changes were small and hard to image. Later developments using extrinsic voltage-sensitive dyes to stain the tissue and photodiode arrays revealed a fast signal that could be imaged and that directly reflected neuronal activity (see reviews1,2).

In 1986, Blasdel and Salama,4 using voltage-sensitive dyes in the monkey striate cortex, a video camera, and a new analysis method, showed for the first time spectacular high-resolution images of OD and orientation columns in vivo in the monkey. They also noted a slow signal during their recording but apparently did not consider the signal to be contributing significantly to the images. Later, Grinvald et al5 showed that this same slow signal, the intrinsic signal, could be used to reveal functional architecture, specifically, cat orientation columns and rat whisker barrels, without using dyes.

Frostig et al12 reported high-resolution (100-150 µm; 200 milliseconds) optical imaging of intrinsic signals in cat and monkey OD and orientation columns using a CCD camera and suggested that the major source of the signal (using 570 nm wavelength light) was due to blood volume changes but was only 3% of the reflection signal. At 600 nm, the signal was probably related to oxygen delivery and/or saturation state of hemoglobin and was 30% to 40% of the reflection signal. They also noted that it temporally preceded the change in blood volume. At the near-infrared wavelength of 810 nm, they were able to image orientation columns through dura, and in the infrared range (930 nm), they were able to image through thinned skull in the cat. Light scattering seemed to play the predominant role in contributing to the signal in the near-infrared

Figure 1. Comparison of temporal and spatial resolution of various brain mapping techniques. MEG indicates magnetoencephalography; ERP, event-related potential; EROS, event-related optical signal; MRI, magnetic resonance imaging; fMRI, functional MRI; PET, positron emission tomography; 2-DG, 2-deoxyglucose. Adapted with permission from Haglund10 and Churchland and Sejnowski.11

<table>
<thead>
<tr>
<th>Terminology of Brain Functional Architecture*</th>
<th>Definition</th>
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<td><strong>Term</strong></td>
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<td><strong>Cortical columns</strong></td>
<td>Many parts of the neocortex have been shown to be composed of microscopic functional units physically represented as discrete groupings of neurons into cortical columns that span the thickness of a cortical layer or the full thickness of the cortex, usually from the surface through most of the immediate underlying layers. Neurons within a column generally all have the same functional properties (ie, they fire in response to the same type of stimulus). In the visual cortex, the stimulus is usually a particular subcomponent of the visual scene (eg, an oriented line, a color, or a direction-specific movement). Neurons also may be grouped according to the eye that is providing the stimulus (OD).</td>
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<td><strong>OD columns</strong></td>
<td>The primary visual cortex contains cortical columns that span the cortical thickness and are composed of neurons responding primarily to input from the right or left eye; †Neurons dominated primarily by the right eye group into columns (right eye OD columns) that run parallel to and alternate with columns containing neurons responding primarily to input from the left eye (left eye OD columns) and form a striplike pattern when seen in a plane from the surface of the cortex (Figure 2A, bottom panel). These stripes are approximately 500 µm wide and were previously detected only by either reconstructed maps of electrophysiologic recordings or postmortem (cortical) tissue analysis of radioactive tracer injections into the eye.</td>
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<tr>
<td><strong>Orientation columns</strong></td>
<td>Orientation columns represent cortical columns of neurons in the primary visual cortex within which all cells will fire primarily only to a line or edge of a specific orientation (10° angle, 20° angle, etc) within part of the visual field of the test subject (eg, a cat) (Figure 2B). Orientation columns generally span the thickness of the cortex. However, they are not lined up in a striplike pattern but rather in a pinwheel-type organization when viewed from the cortical surface.</td>
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<tr>
<td><strong>Whisker barrels</strong></td>
<td>The cortical representation of the receptive field of a single whisker in the rat is physically discernible as an aggregate of neurons called a barrel, located in layer IV of the primary sensory cortex. Each whisker is represented by a different barrel in a 1:1 fashion.</td>
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*CD indicates ocular dominance.
†In layer IVC, neurons respond exclusively to right eye input within right eye OD columns or left eye input in left OD columns.
wavelength and contributed only about 10% of the signal.13

By the use of imaging spectroscopy and laser-Doppler flowmetry in cats, Malonek et al13 found that after visual sensory stimulation, there is an initial increase in deoxyhemoglobin (initid dip) (originating primarily from the capillaries) that later slowly declined and was associated with an increase in total hemoglobin concentration, followed by an increase in cerebral blood flow and oxygenated hemoglobin levels as deoxyhemoglobin levels began to decline. The later increases in blood flow and oxyhemoglobin concentration were considered to be less localizing to the areas of neuronal firing compared with the increase in deoxyhemoglobin concentration, based on earlier observations by Malonek and Grinvald15 that had shown that the increase in oxyhemoglobin was spatially less well registered with activated cortical columns relative to the early increase in deoxyhemoglobin levels. It was concluded that imaging methods based solely on the secondary vascular response, such as positron emission tomography or flow-sensitive magnetic resonance imaging, may offer lower spatial resolution than if they are based on the initial deoxyhemoglobin increase.

METHODS

Most in vivo imaging is currently performed in anesthetized animals, but may also be performed in awake animals and humans. Human optical imaging5 requires some modifications of the animal setup3 (Figure 3). For imaging of intrinsic signals in cats or monkeys, a craniotomy is performed over the area of the brain to be imaged, and a small metal chamber is placed over the site and affixed to the skull with dental cement. The dura is removed in most cases, and then the chamber is filled with silicone oil and sealed with a glass cover. This sealed system helps to dampen arterial brain pulsations that may interfere with imaging. In human studies, a craniotomy is performed, and a glass plate may be placed over the surface of the cortex to dampen pulsations.8 The brain surface is illuminated using flexible liquid light guides supplied by an adjustable direct-current output power supply source and tungsten halogen lamp. Filters are used to produce the desired light wavelength. A green filter (546 nm) is used to obtain baseline images of the cortical vascular surface, and an orange filter (603 nm) is often used for most general imaging. In human studies, this setup has been modified to contain the light source within the operating microscope. The camera (video or CCD) is positioned above the cortex and light guides and is mounted on a vibration-free support. In human studies, the camera is attached to the operating microscope.8 Lenses are attached to the viewing end of the camera and focused on the cortical vasculature to obtain an initial green-light image (Figure 2A-B, top panels). Later, the camera is focused down approximately a few hundred micrometers until the vessels become blurred, and the filter is changed to the imaging wavelength desired.
Sensory stimuli (visual, somatosensory, auditory, etc) are delivered to the animal at a given rate, and the camera collects the reflected light off the brain surface during stimulations. When a video camera is used, a frame grabber digitizes the video signal and sends it to a computer, where the signals are averaged. Various acquisition and analysis procedures may be used. Image enhancement before digitization may be performed by analog differential subtraction of a stored “reference image” from the incoming video image. The signals are later amplified. The differential image may be viewed in real time on a monitor, which helps with rapid information feedback to the investigator. Mapping signals or maps of the intrinsic signals are obtained by various combinations subtracting or dividing the averaged signals from each other. The reflected light signal from the brain surface is very small (0.5%-5%) compared with the background activity; therefore, several stimulations may need to be averaged together to improve the signal-to-noise ratio. To obtain OD maps, signals obtained from right eye stimulation may be divided or subtracted from those obtained from left eye stimulation or from the blank condition. For orientation maps (Figure 2B), vertical grating (vertical lines drifting across the stimulus monitor)–induced signals may be subtracted from horizontal grating–induced signals or from a “cocktail” of combined signals collected in response to several orientations of gratings. Dark areas (light absorption) on the maps are areas of cortex that have been activated in response to the stimulus (Figure 2).3

CLINICAL APPLICABILITY

At present, optical imaging of brain activity is primarily a research technique, but there have been several interesting and revealing human studies. Haglund et al8 obtained optical maps from patients undergoing seizure surgery and correlated them with surface electrical recordings. Maps of the cortical surface were obtained during cognitively evoked functional activity and during electrically evoked epileptiform discharges. In the study of cognitively evoked activity, an attempt to identify Broca’s and Wernicke’s areas was made in patients placed under local anesthesia during testing. Images were collected at rest, while the patient moved his or her tongue, during naming exercises, and during surface stimulation to cause speech arrest. The researchers found that the area involved during naming was different from that during tongue movement. Such studies may help to better define eloquent regions of cortex and subsequently may guide more conservative surgical resection.

In the study of epileptiform discharges, Haglund et al8 found that the recorded optical changes showed a graded response relative to the intensity and duration of the stimulus (surface-stimulating electrodes), which correlated with the electrical changes. In addition, optical changes associated with afterdischarge shifted below baseline in several patients, suggesting a possible inhibitory neuronal population or inhibitory surround or some other cause.

Optical imaging of epileptiform activity in brain slices has been shown to be a useful method for studying various aspects of the spatial and temporal dynamics of seizure spread. In early in vivo studies, it was shown that epileptic foci were not stationary (see reviews1-3). More recently, slice preparations have helped to elucidate the roles of different cortical laminae involved in seizure onset and propagation.8 Brain-slice preparations from human surgical specimens or animals provide an opportunity to study activity in all cortical layers compared with in vivo optical imaging, where imaging is limited to the gyral surface and generally to the first few hundred micrometers of cortical depth from the surface. Such preparations as well as in vivo optical imaging also may be useful for testing the mechanism of action and efficacy of some anticonvulsants and perhaps other medications used to treat other neurologic disorders.

Another potential use for optical imaging in humans is to define tumor margins before and during resection. In an interesting in vivo study by Haglund et al8 in rats, optical imaging of an intravenously injected dye differentiated tumors and their margins from nearby normal tissue with a high degree of sensitivity and specificity. The potential benefit of more accurate surgical resection is undoubtedly desirable.

Although optical imaging may be employed for human psychophysical studies of higher cognitive function, studies are currently restricted to those individuals undergoing neurosurgery procedures. Therefore, such studies might be complemented by the use of event-related optical signal (EROS) imaging using near infrared (NIR) photons. This technique is now used to image evoked higher cortical activity through the skull in humans and...
currently yields a spatial resolution of a few millimeters (significantly less than that of optical imaging but similar to that of functional magnetic resonance imaging) and a temporal resolution of about 20 milliseconds (camera sampling rate).^{16}

**NEUROSCIENCE APPLICATIONS**

Optical imaging of brain signals has already yielded many exciting new insights into the functional architecture of the cerebral cortex. In the study by Blasdel and Salama of OD and orientation columns in monkeys, the authors demonstrated that orientation column functional architecture was not organized in linear slabs of alternating orientation columns as had earlier been suggested by Hubel and Wiesel (although they speculated that the columns were likely not sharply linear in organization). Instead, they had more of a rosette appearance. That study and another one in cats by Bonhoeffer and Grinvald led to the demonstration of a more pinwheel type of organization of orientation columns.

At present, it is well accepted that brain plasticity is no longer solely restricted to the critical period of development in many organisms, and methods to measure spatial aspects of receptive field reorganization or expansion have often been limited to painstakingly slow single-electrode recordings. Optical imaging has proven to be a useful tool in measuring dynamic changes in large populations of neurons at one time, and hence is particularly helpful in investigations not only of temporal but also spatial aspects of plasticity. Polley et al made an intriguing observation in adult rat whisker-barrel cortex, in which they showed plasticity of the cortical representation of whisker barrels as a function of sensory deprivation and novel environmental stimuli. In that study, they removed all large whiskers on the rat except one and observed a large-scale expansion of the remaining whisker's functional cortical representation. However, when the animal was removed from its cage and put into a new environment to explore it briefly, there was large-scale contraction of the barrel's cortical representation. Contraction and expansion of the cortical whisker barrels reversed upon growth of the whiskers.

Unpublished observations by Szapiel et al (Figure 2C), have revealed a striplike pattern of activity in the posterior parietal lobe of an awake rhesus monkey in response to an expanding optic flow stimulus, suggesting a previously unseen functional architecture for this type of stimulus using optical imaging techniques. Optic flow is the perception of motion as we move forward or backward through the environment. When moving forward, objects tend to flow past us in an expanding pattern from a central focus; moving backward, the pattern appears to be one of contraction. A moving pattern of expanding or contracting dots emanating from or flowing toward a central point on a computer screen can simulate optic flow when viewed by a subject at a specific distance from the screen. The inferior parietal cortex of the rhesus monkey has been shown to contain neurons that respond to visual optic flow.

High-resolution optical imaging of intrinsic signals and real-time optical imaging with voltage-sensitive dyes in vivo and in vitro continue to advance our understanding of basic brain structure and function, and as a result, provide another important tool with which to understand and treat neurologic disorders.

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