Chapter 17
Intrinsic Optical Signal Imaging of Normal and Abnormal Physiology in Animals and Humans—Seeing the Invisible

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INTRODUCTION

Surgeons in general and neurosurgeons in particular are extremely dependent on their sense of vision. After the advances in antiseptic and anesthetic technique, which were critical to the establishment of surgery as a field in medicine, most recent advances in the field of neurosurgery have arisen from improvements in the surgeon¡¦s ability to see the relationship between normal and abnormal anatomy and to translate these images into the operating room as the basis for a therapeutic intervention. Although the microscope and endoscope allow us to see gross anatomic structures with finer detail, the implementation of image-based stereotaxy and intraoperative magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound permits us to anticipate anatomy that cannot be observed grossly.

Neurosurgeons, however, do not just operate on anatomy, but also on physiology. The locations of the specific areas of the brain responsible for movement, sensation, vision, and language, or the site of an epileptic focus, are generally not immediately apparent to gross visual inspection or even to high-resolution anatomic imaging, such as MRI. Likewise, cerebrovascular hemodynamics, such as cerebral blood flow (CBF), cerebral blood volume (CBV), or hemoglobin oxygenation may be focally impaired in certain disease states, which may also not be apparent from anatomic imaging. Hence, techniques that permit the neurosurgeon to map normal and abnormal physiology are critical to the development of our field, particularly if they can be done in real time in the operating room.

Currently, we map cortical electrophysiology intraoperatively using arrays of electrodes placed on the surface or into the depths of the brain. Blood flow or oxygenation can be measured with small, handheld probes. The main disadvantage of these techniques is in sampling because information is recorded from only a small region of adjacent space. Extraoperatively, several perfusion-based imaging modalities exist to measure physiology, such as positron emission tomography (PET), functional MRI (fMRI), single-photon emission tomography (SPECT), or xenon CT. These techniques are not only useful in mapping blood flow and oxygenation but also electrophysiology, on the basis of a presumed understanding of the coupling and uncoupling of neuronal activity and perfusion. Although these techniques can sample the brain widely, both on the surface and at a depth, they have limited spatial and temporal resolution, at best on the order of a few millimeters or a few seconds. In addition, although the coupling between neuronal activity and perfusion is well studied after normal physiological events, such as somatosensory or visual stimulation, little is known about this relationship after an abnormal event, such as epilepsy.

This chapter summarizes our laboratory¡¦s work using a technique called optical recording of intrinsic signals (ORIS). As we will explain, ORIS currently has the highest combined spatial and temporal resolution of any technique for mapping cortical physiology without the use of potentially toxic dyes, combined with the advantage of sampling large areas of cortex simultaneously (6, 65). We have found ORIS extremely useful in gaining a better knowledge of the relationship between an abnormal cortical physiological event, such as epilepsy, and the brain¡¦s perfusion and

oxygenation response. These findings are critically important to our understanding of the perfusion-based imaging techniques that have found their way into clinical practice and imply an understanding of this relationship that has not previously existed. We have also translated these techniques into the neurosurgical operating room to map both normal and abnormal cortical physiology, which is increasing our ability to see what has heretofore been invisible to the neurosurgeon¡¦s ever-increasing gaze.

THE INTRINSIC OPTICAL SIGNAL (IOS)

The IOS is a small change in the absorption (or reflection) of light that occurs in neuronal tissue when neurons are activated. These changes can be recorded from a variety of preparations, ranging from a single neuron preserved in vitro (31) to the human brain in the neurosurgical operating room (65). ORIS has been used extensively to map static functional architecture, such as orientation and ocular dominance columns in visual cortex, leading to major discoveries, such as the pinwheel organization of orientation columns (Fig. 17.1). The origins of the IOS are multiple, because neuronal activity induces a cascade of events in the surrounding tissues, each of which can influence the reflection of light. The real power of IOS arises from the fact that, depending on the wavelength of light, the IOS can separately measure changes in CBV, hemoglobin oxygenation, or light scatter (LS) with a temporal resolution of approximately 100 ms and a spatial resolution of <200 ƒÝm (6). At isosbestic wavelengths of hemoglobin, where oxygenated hemoglobin (HbO2) and deoxygenated hemoglobin (Hbr) reflect light equally (525, 545, 570.5, and 583 nm; corrected for path length) (68), ORIS measures total hemoglobin (Hbt), which is directly proportional to CBV and CBF, assuming that the concentration of red blood cells remains constant (49, 52). At higher wavelengths (600¡V650 nm), the majority of the signal arises from the oxygenation state of hemoglobin, because Hbr absorbs light with three times the absorption coefficient of HbO2 (46, 61). Hence, a decrease in light reflection indicates an increase in Hbr. At wavelengths >650 nm, the signal from LS becomes progressively more significant and ultimately dominates the IOS in the near-infra red region (>800 nm), particularly in avascular preparations (46). The LS component of the signal arises from a combination of movement of sodium and potassium ions in and out of both neurons and glia, associated fluid shifts, and increases and decreases in the volume of the extracellular space as well as the morphology of the vasculature (6, 31, 61). Although LS is present at all wavelengths, the contributions of the signal arising from hemoglobin decrease with increasing wavelength. Only in bloodless preparations can a pure LS signal can be recorded.

COUPLING AND UNCOUPLING BETWEEN NEURONAL ACTIVITY AND PERFUSION

The idea that neuronal activity, which causes an increase in local cerebral metabolic rate (CMRO2), would also lead to a focal increase in cerebral perfusion is based on the work of Roy and Sherrington (59). As a result, techniques that are sensitive to perfusion can then be used to map neuronal activity. More recently, Fox and Raichle demonstrated that increases in CBF, which occur 1 to 2 seconds after the neurons become active, are far greater than increases in CMRO2 (20). This mismatch, or uncoupling of metabolism and blood flow, leads to a local increase in HbO2, which is the basis of the blood oxygen level¡Vdependent (BOLD) signal used for brain mapping with fMRI, because differences in the paramagnetic properties of oxygenated and deoxygenated hemoglobin can be measured with high magnetic fields (54) (Fig. 17.2). However, a great deal of controversy still surrounds the first few hundred milliseconds after neurons become active. Some investigators have been able to measure an early decrease in hemoglobin oxygenation (increase in Hbr) called the initial dip, which is spatially and temporally more focally related to the populations of active neurons with the use of techniques such as ORIS (21, 68), imaging spectroscopy (46,

50), oxygen-dependent phosphorescence quenching (79), and fMRI at 1.5 T and 4 T (Fig. 17.2) (37, 43). Nevertheless, there is also ample evidence of an absence of an initial dip using these same techniques and, thus, its existence remains controversial (42, 69). Other explanations for these inconsistent results point to the dependence of the initial dip on anesthetic techniques, pO2, analysis algorithms, as well as stimulus and species dependence (42, 80).

The putative existence of this initial dip in hemoglobin oxygenation is of critical importance. As a mapping signal, the early CMRO2-related increase in Hbr may have a more precise spatial correlation with the population of active neurons than the later CBF-related increase in HbO2 that forms the basis of the BOLD mapping signal (21, 46, 79). If this is the case, fMRI may need to focus attention on earlier events to avoid potentially mislocalizing cortical activity. On the other hand, another potential mapping signal that is measurable both with ORIS and fMRI arises from very early activity-related increases in CBV, which were initially thought to be less localizing than the initial dip (21, 46). Recent animal data, however, has shown that increases in CBV may be highly localized within the first 2 seconds after the electrophysiological event, potentially offering a higher signal-to-noise ratio (SNR) than the initial dip (52, 67, 68), especially if one can separately identify the signal arising from microvasculature rather than the macrovasculature (81). Despite extensive literature on the relationship between perfusion and neuronal activity after normal cortical activity, little work has been done on the hemodynamic events surrounding abnormal activity, such as epilepsy.

IOS AND EPILEPSY

Epilepsy is a clinical term referring to a disease involving recurrent seizures that effects between 1 and 2% of the population of the United States (30). Seizures consist of the paroxysmal, synchronous, rhythmic firing of a population of pathologically interconnected neurons capable of demonstrating high-frequency oscillatory activity (7). These events are caused by an imbalance in excitatory and inhibitory mechanisms leading to both hypersynchrony and hyperexcitability (66). Surface recordings from patients with chronic epilepsy or intracortical field potential (f.p.) recordings adjacent to experimentally induced epilepsy in laboratory animals show abnormal paroxysmal events in a large population of neurons called interictal spikes (IISs). The IIS generally consists of a high amplitude surface negativity (1¡V5 mV) lasting 50¡V200 ms followed by a slow wave or afterhyperpolarization (AHP) with no behavioral correlate. The transition to an ictal event, or seizure, occurs when the AHP gradually disappears and is replaced by further depolarization (15).

Previous investigations in the relationship between epileptiform events and perfusion or metabolism have demonstrated contradictory results in both animals and humans using autoradiography, PET, and fMRI; all of which are techniques with limited temporal and spatial resolution (1, 33, 38, 53, 55, 75, 77). Although an increase in perfusion is universally demonstrated, some studies find that perfusion oversupplies metabolism (3, 41, 53, 77), whereas other studies demonstrate the opposite effect, namely, inadequate perfusion to meet metabolic demand (33, 38, 55, 75).

The IOS has been previously applied to the study of epileptogenesis in in vitro slices of cortex (32), isolated guinea pig whole brains (18), and in in vivo rats (11), ferrets (63, 64), and humans (28) (Fig. 17.3). However, these studies were all performed at near infrared wavelengths sensitive mostly to LS. Although these studies clearly demonstrated that the IOS can identify the site of an IIS or ictal onset zone (64) as well as areas of surround inhibition (64), and

may even be sensitive to preictal phenomenon that can be useful in predicting the onset of seizures (11), the relationship between epileptiform events and CBV or Hbr has not been investigated with the temporal and spatial resolution provided by the IOS. An epileptiform event, either interictal or ictal, involves the synchronous, rhythmic activation of a large population of neurons and an enormous increase in CMRO2 (19). One central concern of our laboratory has been the coupling and uncoupling between CMRO2 and CBV and Hbr in the face of such supranormal metabolic demand.

EXPERIMENTAL METHODS

Our laboratory uses a variety of preparations and models of in vivo epileptogenesis. All experiments were approved by the institutions¡¦ Institutional Animal Care and Utilization Committee (IACUC) or Institutional Review Board (IRB) committees. To induce acute IISs, we generally use focal iontophoresis of 5 mmol/L of bicuculline methiodide (BMI) in 165 mmol/L of NaCl, pH 3.0, into layers II to III, resulting in periodic IISs. Acute ictal events, on the other hand, are easily induced with focal injection of 0.5 ƒÝl of 25 mmol/L of 4-aminopyridine (4-AP). Typical events last approximately 90 seconds with a 2-minute interseizure interval. We have used both models in rats and ferrets, the latter species used to investigate the relationship between epileptiform events and visual architecture. Rats are induced with ketamine and xylazine and then maintained with 1.25 g/kg of urethane, whereas ferrets were maintained with 0.9% halothane. Electrocardiogram (EKG), pCO2, pO2 and temperature are continuously monitored and stably maintained.

Imaging is performed with a commercially available imaging system (Optical Imaging Inc., Germantown, NY) (Fig. 17.4). The bone is thinned (rat) or a craniotomy is performed and the dura opened (ferret and human). Cranial stabilization requires either a stereotactic frame (rat and ferret) or a Mayfield head hold (human). Cortical pulsations are limited with the use of 0.2% agar and coverslip (rat and ferret) or a glass footplate (human). The cortex is illuminated using either fiberoptic light-guides (animal) or a ring illuminator (human), filtered to measure CBV (546 nm), Hbr (605 nm and 630 nm), or LS (700 nm or 707 nm). Frame duration varies depending on the experiment, from 100 to 600 ms with acquisition frequencies of 10 to 1.6 Hz. Events are visualized by dividing the frame that occurs before the event by subsequent frames. Although IISs can be visualized after a single event, averaging is used to increase the SNR ratio (Fig. 17.5). No averaging is used for ictal events. Images are smoothed with a high-pass filter.

THE EPILEPTIC DIP

Using ORIS at wavelengths sensitive to hemoglobin oxygenation (605 nm and 630 nm), our laboratory has recently shown that IISs and ictal events induce a profound and immediate (<100 ms) increase in Hbr (2, 74). We use the term epileptic dip to refer to this decrease in hemoglobin oxygenation elicited by the increase in metabolic demand associated with synchronized epileptiform population activity. Using focal iontophoresis of BMI, a ƒ×-aminobutyric acid (GABA)-A antagonist, into rat neocortex as a model of IISs, we have demonstrated that the increase in Hbr lasts as long as 3 to 4 seconds (Fig. 17.6). With microinjection of 4-AP, also in the rat, we can elicit ictal events that last several hundred seconds (74). We find a similar increase in Hbr lasting throughout the length of the ictal event (Fig. 17.7). From this data, we conclude that, unlike normal physiological events in the brain, epileptiform events raise CMRO2 beyond the capabilities of the brain to sufficiently perfuse the activated neurons with oxygenated blood (Fig. 17.8). If the epileptiform event is short, such as occurs in an IIS, the increase in CBV will eventually furnish enough oxygenated hemoglobin that a BOLD signal will be found several seconds after the event. However, if the IISs occur

with a high enough frequency, or transition to an ictal event, then the increase in Hbr persists and a localized BOLD signal is not observed (Fig. 17.8).

There are several implications to this discovery. First, it directly contradicts most fMRI studies of epileptiform events, which have generally shown an increase in BOLD signal, implying a focal overabundance of HbO2 (3, 41, 53, 77). However, these fMRI studies lack the spatial and temporal resolution of ORIS. Our data also imply that fMRI mapping of epileptiform events may mislocalize the ictal or interictal event if a region of positive and not negative BOLD is recorded. We propose that with sufficient magnet strength, fMRI studies will ultimately confirm our findings, particularly if epileptiform events of longer duration or involving synchronization of a larger pool of neurons are examined. Indeed, in one fMRI study in animals, more frequent and intense epileptiform events, such as γ-butyrolactone (GBL)-induced absence seizures showed a more prominent negative BOLD signal (76). Likewise, fluorodeoxyglucose (FDG)-PET of acute rat IISs do reveal hypermetabolism within the focus, particularly at high spike-frequency rates (29) and hypometabolism in the surround (8), whereas in more chronic models with lower spiking frequencies, only hypometabolism is apparent in both the focus and surround, as is found in human epilepsy (26). Our data also confirms and extends previous autoradiographic studies in animals, which find that the increase in perfusion associated with status epilepticus (SE) may not be adequate to match the persistent increase in CMRO2 (33, 55, 75). However, these studies have not directly demonstrated that SE induces ischemia, which is clearly observed in our data. These results may partially explain the extent of neuronal injury associated with SE (51).

CBV MAPPING

Recent experimental evidence has shown that an increase in CBV may be quite focal within the first 2 seconds after the onset of normal physiological neuronal activity (52, 67, 68, 81). The CBV response after epileptiform events, however, has only been investigated using autoradiography (1, 55, 75), SPECT (78), and fMRI (3, 41, 53), which lack high temporal resolution. Using ORIS at 546 nm, we have shown that as early as 100 ms after an epileptiform event, one can record a focal increase in CBV that is as highly localized as the increase in Hbr or change in LS (Fig. 17.6) (74). However, the amplitude of the signal is not as large as the Hbr signal within the first 2 seconds and at later times, when the amplitude of the CBV signal is higher, the area of the signal is more widespread, indicating that perfusion has spatially oversupplied the region of neuronal activity, rendering the CBV signal less useful as a mapping signal (Fig. 17.7). There are several important ramifications of this finding. First, fMRI, which is sensitive to CBV, might be useful in mapping ictal onsets, as has already been shown for normal sensory physiology, if the first few seconds can be isolated (47). Second, one of the controversies surrounding the initial dip is that a complimentary reduction in HBO2 is not also recorded in the first few seconds after neuronal activation, which has lead several investigators to hypothesize a simultaneous highly localized increase in CBV (46). We have now clearly demonstrated this early increase in CBV after epileptiform events. Measuring whether a smaller increase in CBV is also present immediately after normal sensory processing may require instruments that are more sensitive, but our results suggest that a more aggressive search for this phenomenon may be fruitful. Finally, some investigators have suggested that the CBV signal may be more localizing than the Hbr signal for mapping ictal events in humans (27). Our data does not quite agree because the focality of the CBV signal diminishes rapidly after the onset of the epileptiform event.

SURROUND INHIBITION AND INVERTED IOS

In the late 1960s, using focal injection of penicillin into cat neocortex, Prince and Wilder (58) first described a ring of inhibitory postsynaptic potentials (IPSPs) surrounding the epileptic focus using intracellular recording in vivo. They coined the term “inhibitory surround” to describe the phenomenon, a presumed ring of neuronal inhibition surrounding an epileptic focus and restricting horizontal propagation. These findings were later confirmed by other investigators using different models of acute animal epilepsy (23). It is unclear whether this phenomenon is inherent to epileptogenesis or particular to certain animal models of epilepsy because surround inhibition has not been found in chronic animal models or human epilepsy, although little electrophysiological data exists (34, 57). SPECT and PET data in humans do reveal an area of hypoperfusion and hypometabolism surrounding human foci, but the relationship to underlying neuronal activity is unclear (16, 78).

In several studies that use the IOS to map epileptiform events, an inverted optical signal is often recorded surrounding the epileptic focus, raising the possibility that the inverted optical signal may correspond with neuronal inhibition. This was first described by Haglund et al. (28) who were recording 690 nm after electrically triggered afterdischarges in human cortex. Although this wavelength is most sensitive to LS, they attributed the inverted signal to shunting of blood from the adjacent brain to the epileptic focus. More direct evidence that the inverted IOS may correlate with neuronal inhibition came from a study by Schwartz and Bonhoeffer (64) in ferret visual cortex, where an inverted IOS recorded at 707 nm spatially overlapped with a decrease in the firing of extracellularly recorded neurons. Support for this theory emerged from IOS recordings from mouse visual cortex at 707 nm, where, again, an inverted optical signal correlated spatially with a decrease in firing of extracellularly recorded neurons (62).

By recording from interictal foci at 546 nm, 605 nm, and 700 nm, our laboratory has directly measured CBV, Hbr, and LS from the area surrounding an epileptic focus (74). We found that an inverted signal is apparent as early as 100 to 200 ms after the IIS at all measured wavelengths and that this signal peaks at 1.5 s, indicating that there is a simultaneous decrease in CBV, a decrease in Hbr, and a decrease in LS surrounding an acute interictal focus (Fig. 17.6). Thus, we have directly shown that in the cortex, surrounding an epileptiform focus, blood indeed is shunted into the focus and oxygenation increases. Because CBV decreases in the surround, the simultaneous decrease in Hbr cannot be attributed to an influx of oxygenated hemoglobin, as would occur with the BOLD signal. A more likely explanation is a decrease in neuronal activity and CMRO2, as occurs in an electrophysiological inhibitory surround.

We have also found a second wavelength-dependent peak to the inverted optical signal that occurs later (peaks at 3 s after IIS) and arises within the focus and draining veins in a subset (2 out of 10) of the animals whose IISs occur at a slower frequency (0.19 ± 0.06 Hz versus 0.33 ± 0.08 Hz; P = 0.08) (Fig. 17.9)(74). This later inverted signal is consistent with the BOLD signal recorded with fMRI arising from a later influx of HbO2. Thus, a short duration epileptiform event, such as an IIS, will elicit a BOLD signal from the focus and draining veins if the inter-event interval is long enough to permit the increase in perfusion to oversupply the metabolic demand of the focus (Fig. 17.8) (74).

In the region of cortex surrounding an ictal focus, our multiwavelength recordings also show decreases in CBV, Hbr, and LS (2). However, because the area of perfusion oversupplies the ictal focus, there seems to be two separate rings of inverted perfusion surrounding the ictal focus. In the inner ring, we find an increase in CBV and a decrease in Hbr, whereas, in the second ring, we find both a decrease in CBV and a decrease in Hbr (Fig. 17.10) (2). One explanation for these two rings is an uncoupling of oxygenation and perfusion in the surround. If pyramidal cells are inhibited and less metabolically active, one would expect a decrease in Hbr, as we have shown. However, because the increased CBV to the metabolically active focus is not spatially restricted, we find a paradoxical increase in CBV

in regions immediately surrounding the focus, where pyramidal cells are inhibited. Further outward, in the outer surround beyond the region where CBV overspills the focus, we have identified decreases in both CBV and Hbr. In this second surround, blood may be shunted to the more metabolically active focus. Similar regions of decreased perfusion have been identified in humans surrounding an ictal focus using ictal SPECT (78). Alternative explanations include the possibility of degrees of inhibition around the focus or variability in the metabolic demand of the interneurons responsible for the IPSPs. Testing these hypotheses will require simultaneous physiological recording from multiple neuronal populations with either electrode arrays or voltage-sensitive dyes.

RELATIONSHIP WITH UNDERLYING FUNCTIONAL ARCHITECTURE

Partial onset neocortical epilepsy initiates in a focal region of cortex and then propagates horizontally. The interactions between epileptiform events and the columnar architecture of the cortex, as well as the structure of long- and short-range excitatory and inhibitory connections, have been widely debated. Some authors have postulated that the recurrent excitatory connections within the cortical column form the basic unit of epileptogenicity (22, 24). Others have demonstrated a nonuniform horizontal propagation velocity in both in vitro and in vivo preparations (13), which is thought to reflect variations in the density in long-range horizontal excitatory connections (14).

We have directly investigated the relationship between epileptiform events and functional architecture using ORIS to simultaneous map an IIS focus as well as the architecture of orientation, spatial frequency, and ocular dominance columns within the ferret visual cortex (63). By placing our BMI iontophoresis pipette within a known orientation column, we were able to preferentially trigger IISs with visual stimulation of a similar orientation. Hence, we were able to show that selective behavioral stimuli can elicit epileptiform events within certain susceptible areas of cortex (Fig. 17.11). These findings demonstrate an intracortical etiology for the phenomenon of pattern-sensitive epilepsy, in which clinical epileptic events are triggered by patterned visual stimuli (5). In addition, we were able to use ORIS to map the architecture of visual cortex within, and adjacent to, an interictal focus to examine the dynamic effect of epileptiform events on the functional architecture (65). In these experiments, we mapped the architecture of the orientation columns before inducing IISs, began iontophoresing BMI into a particular orientation column, and again mapped the orientation columns during IIS events. We found that there was a gross distortion of the architecture of the orientation columns within the focus, and that the orientation preference of the neurons in adjacent columns shifted to prefer the orientation of the column into which the BMI was injected (Fig. 17.11). In the surrounding cortex, however, the orientation preferences and architecture of the orientation columns were completely unaffected (Fig. 17.11). When the iontophoresis was reversed and the IISs ceased, the orientation preference within the focus returned to its baseline (Fig. 17.11). From these experiments, we concluded that interictal epileptiform events have a profound but reversible effect on the functional architecture of the brain. This transient distortion of columnar circuitry is regionally limited to the population of neurons participating in the epileptiform event, with little impact on surrounding neurons. These results shed light on the cognitive behavior of patients with frequent interictal events. Although epilepsy leads to chronic cognitive deterioration (44), patients are able to function well despite frequent interictal events. For example, pattern-sensitive epileptics have normal vision interictally (82). However, subtle testing may reveal deficits in brain function, specifically in the areas where the spikes are occurring, potentially caused by transient ischemia arising from the epileptic dip (4).

HUMAN IMAGING

Because ORIS has the highest combined spatial and temporal resolution of any brain mapping technique that does not use potentially toxic dyes, there has been considerable interest in using this technique to map human brain (56). Although it is possible to use near infrared light through the intact cranium (25), the spatial resolution is limited unless a craniotomy is performed. Hence, several attempts have been made at human brain mapping during neurosurgical procedures. The IOS was first used intraoperatively by MacVicar et al. in 1990 (45) for the imaging of stimulation-evoked cortical activation. This study was soon followed by that of Haglund et al. (28) who imaged both stimulation-evoked epileptiform afterdischarges and cognitively evoked functional activity, such as activation of Wernicke's and Broca's areas during language tasks. Another group at the University of California, Los Angeles, lead by Arthur Toga has published several articles on intraoperative human imaging of somatosensory and language cortex (10, 56). Other groups have also imaged somatosensory cortex, including Shoham and Grinvald (70), Sato et al. (60), and Schwartz et al. (65).

Although IOS of human cortex in the operating room is quite feasible, the signal is not as robust as in the laboratory and the spatial resolution is lower because of several technical challenges and large sources of noise (10, 56, 60, 65, 70). The major sources of noise include motion of the cortex induced by heartbeat and respiration, as well as a 0.1-Hz vasomotor signal, each of which change the reflected light signal with a different periodicity (36, 48, 65). In addition, because of the time constraints of the operating room, the environment is more difficult to control, resulting in larger fluctuations in ambient light (28, 65) and anesthesia. Various mechanisms have been developed to compensate for these sources of noise. Image acquisition can be synchronized to the cardiac and respiratory cycles (10). The cortex can be stabilized with a glass footplate (28, 60, 65, 70), and post hoc algorithms can also be applied to remove noise from the imaging data. These can include warping algorithms (10, 28), or, if a sufficiently long series of images can be obtained, an inverse fast Fourier transform (FFT) algorithm can be used to remove periodic fluctuations from the time course of the signal (36).

We developed a camera holder specifically for intraoperative ORIS and have used the system to map somatosensory cortex as well as bipolar cortical stimulation (65). Most recently, our laboratory has been interested in analyzing whether the initial dip in hemoglobin oxygenation, which we have clearly shown to be present after epileptiform events, also occurs in the human brain (73). To address this question, we performed bipolar stimulation of the human cortex at varying amplitudes and mapped the IOS at wavelengths sensitive to CBV (546 nm) and Hbr (605 nm). Bipolar stimulation, like epileptiform activity, elicits synchronized depolarization in a localized population of neurons that is supraphysiological. We have found that after bipolar stimulation there is a rapid (<200 ms) increase in both CBV and Hbr localized to the region beneath the electrodes (73). Although the Hbr signal remains focal for several seconds, the CBV signal soon spreads trans-sulcally to adjacent gyri (Fig. 17.12). A late BOLD signal is also recorded that is poorly localized to the site of stimulation. In addition, we have found that the size of the initial dip varies nonlinearly with the amplitude of the stimulation. These results mirror our results from measuring IOS after epileptiform events and likely represent the fact that bipolar cortical stimulation is a supraphysiological activation of the cortex, not unlike epilepsy. Several conclusions can be drawn from these results. First, the initial dip in hemoglobin oxygenation is clearly present, even in human cortex, after high levels of neuronal activation. Whether it is present after normal physiological activation is still under debate. One possible physiological mechanism for the obvious dip in oxygenation in our studies may be that there is a tissue oxygen buffer, which must be overcome before hemoglobin oxygenation decreases (9). Thus, experiments involving normal sensory processing that fail to find the initial dip may either require instruments that are more sensitive to record a very small initial dip, or the initial dip may not be present until a certain threshold of activation is reached. Second, we have confirmed that a focal increase in

CBV occurs rapidly at the site of neuronal activation in the human. This signal may be useful in human brain mapping if it can be isolated to the first 1 to 2 seconds after cortical activation. At later time points, this signal will not be well localized. Finally, the BOLD signal, or late hyperoxygenation from the delayed increase in CBF and CBV, is not as good of a localizing signal in the human as the initial dip or early CBV signal. Thus, our findings in humans are in accordance with our animal data.

LIMITATIONS—SEIZURE PREDICTION AND EVOLUTION OF SEIZURE

Although the IOS is extremely useful to address certain questions, there are also significant limitations to its use. It must never be forgotten that the IOS does not directly measure electrophysiology, but, rather, measures other physiological events that are indirectly linked with electrophysiological events. For example, although the temporal resolution of the onset of certain components of the signal is potentially quite rapid (likely <100 ms with a sufficiently sensitive camera and adequate signal processing), the offset of the signal seem s to be quite slow. Hence, mapping dynamic events, such as epilepsy or spreading depression (as opposed to static functional architecture, such as cortical columns), is difficult during the course of the event because rapid changes in the underlying electrophysiology will be hidden by the long offset of the signal responding to an earlier electrophysiological event.

One illustrative example of this limitation in the IOS can be found in the 4-AP model (2). We have found that ictal events arising from focal injection of 4-AP sometimes start as low-voltage fast activity (LVFA) and sometime as spike and wave activity (SW), both of which also occur in humans (17). LVFA, also called the “recruiting rhythm,” is thought to represent periods of disinhibition, on the basis of in situ human hippocampal single-unit recordings (17, 39). SW, on the other hand, involves high-amplitude repetitive spike-and-wave events that can occur in low frequencies (1–2 Hz) or at higher frequencies (10–20 Hz) in the few seconds before or immediately at seizure onset (39, 71). This type of ictal event has been correlated with an increase in inhibitory tone on the basis of single-cell recordings from the human hippocampus (35). Supporting the idea of increased inhibition in SW seizure onset is the wave portion of the spike-and-wave complex, which has been shown to correlate with neuronal hyperpolarization (72).

Despite these dramatic differences in the electrophysiology of LVFA and SW ictal evolution, the IOS is identical during both events and does not change when a seizure begins as LVFA and then evolves into SW (Fig. 17.13). This limitation in the ability of the intrinsic signal to rapidly reflect alterations in the evolution of a dynamic electrophysiological event is a significant limitation in the ability of the IOS to map the evolution of a seizure. Some investigators have proposed that the IOS may be useful at mapping pathways of propagation through the brain (27), and our data indicates that, although sensitive to ictal onset, the IOS may not be an ideal reflection of cortical activation as the seizure develops.

In addition, claims have recently been made that the IOS may be useful at predicting the onset of a seizure, implying that perfusion-related events may actually precede the electrophysiological ictal onset (11). These changes in perfusion and metabolism might either reflect electrophysiological events, which have been shown to predict seizure onset (40), or, even more intriguingly, perhaps be independent of any membrane potential changes that might be recorded with electrodes. However, in the rat 4-AP model, we have not found any preictal alterations in the IOS, which might predict seizure onset, other than a demonstration of a build-up of IISs. In the previous study by Chen et al. (11), the preictal optical changes, measured only at 850 nm, occurred in the cortex contralateral to the f.p. electrode and penicillin focus, therefore, no electrophysiology was available adjacent to the area where the optical

change was measured. Hence, the presumed preictal optical changes may have been IISs.

CONCLUSIONS

Using optical recording methods, we can sample changes in CBV and hemoglobin oxygenation from large areas of cortex simultaneously with high spatial and temporal resolution in both animals and humans. We have found that epileptiform events induce a significant, focal decrease in hemoglobin oxygenation, consistent with the initial dip, whose presence is variably found after normal physiological events. For this reason, we have used the phrase epileptic dip to describe this phenomenon. Focal ischemia during longer epileptiform events may explain the neuronal injury associated with status epilepticus. We have also found that a focal increase in CBV occurs quite early after the onset of neuronal activity (<200 ms) and is a useful signal for mapping the onsets of epileptic events, but rapidly spreads to a large nonlocalized region of cortex. The BOLD signal, or late increase in hemoglobin oxygenation, is only found after short and infrequent interictal events and is less spatially localized to the region of active neurons than the initial dip or early CBV signal. All results were confirmed in human brain using bipolar cortical stimulation. Human brain mapping with ORIS is quite feasible and useful in the neurosurgical operating room. A detailed understanding of the nature of the IOS at different wavelengths, as well as its sensitivity to underlying electrophysiological events and its limitations, are required to implement this powerful technique in neurosurgical practice.

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Fig. 17.1 Optical imaging of intrinsic signals reveals the functional architecture in ferret visual cortex. A, blood vessel pattern of the surface of the visual cortex. B, angle map of the orientation columns in visual cortex obtained with IOS imaging at 707 ± 10 nm demonstrates the pinwheel organization of the orientation columns. Each color represents the preferred orientation of the population of neurons in that area of cortex. C, ocular dominance columns in the same region of cortex are obtained by alternating eye shutters to stimulate each eye independently. Dark areas represent regions preferring right eye stimulation, whereas light areas represent region preferring left eye stimulation. The ocular dominance columns in the ferret are variable and irregular. Scale bar: 1 mm. From, Schwartz TH: Optical imaging of epileptiform events in visual cortex in response to patterned photic stimulation. Cereb Cortex 13:1287–1298, 2003 (63).

Fig. 17.2 Perfusion/oxygenation events during normal cortical processing. A, at rest, oxygenated hemoglobin (HbO2) in red blood cells flows from arterioles through capillaries to venules to provide oxygen for neurons. B, after normal physiological neuronal activation (darkened pyramidal cells), there is an increase in neuronal metabolism that may cause a focal increase in deoxygenated hemoglobin (Hbr), also known as the initial dip, which is seen as an decrease in reflection of light at wavelengths sensitive to hemoglobin oxygenation. Whether an equally early increase in CBV occurs is unknown. C, several seconds after neuronal activation, an increase in perfusion and CBV brings an oversupply of HbO2, which forms the basis of the BOLD signal imaged with fMRI. This signal appears in the draining venules, and may be less well localized to the population of active neurons.

Fig. 17.3 Optical imaging of interictal focus in ferret visual cortex. A, blood vessel pattern on the surface of ferret visual cortex and relative locations of the f.p. and electrocorticography (ECoG) electrodes and iontophoresis pipette (BMI). B, simultaneous optical signal, f.p., and ECoG recording show that each interictal spike has a discrete optical correlate. Note that the ECoG, located beyond the limits of the optical signal, does not record the interictal events, supporting the conclusion that the optical signal indicates the spatial limits of the electrophysiological event. C, optical maps, each averaged during 1 minute of recording (approximately 21 spikes). During the earliest, small amplitude IISs, the mean area of the focus for all experiments was 0.12 ± 0.02 mm2, with a minimum of 0.08 mm2. The area of the focus increased in size during the next several minutes and finally stabilized at a mean size of 2.84 ± 1.59 mm2, corresponding to an increase in the amplitude of the f.p. spike. The area of the IIS was derived from the BD maps by thresholding to a pixel value one standard deviation (SD) above the pixel values from the area of the focus during control conditions. Notice that the negative optical signal in the surrounding cortex also increases in intensity and area as the focus develops. Scale bar: 1 mm. From, Schwartz TH, Bonhoeffer T: In vivo optical mapping of epileptic foci and surround inhibition in ferret cerebral cortex. Nature Med 7:1063–1067, 2001 (64).

Fig. 17.4 Schematic diagram of the experimental setup for in vivo optical imaging in the rat neocortex. At bottom, the rat brain is exposed and one side of the cranium over the neocortex is thinned. A small hole is made in the thinned cranium and the dura below, and a field potential electrode is inserted, along with a second electrode, through which a pharmacological agent may be injected to cause ictal or interictal events. The field potential signal is continuously recorded. During imaging, images are collected by the charge-coupled device (CCD) camera placed above the thinned cranium. A tightly time-locked pulse from the imaging computer is fed into the computer that records the electrophysiology, so that the imaging and the interictal or ictal events can be correlated in time during off-line analysis.

Fig. 17.5 Spike-triggered image division of single or multiple interictal spikes. A, image obtained at 546 nm shows blood vessel pattern seen through a thin layer of cranium. BMI, iontophoresis bicuculline electrode; F.P., field potential electrode. B, stable, well-formed biphasic IISs were induced at regular intervals with focal iontophoresis of BMI. ORIS spike-triggered map at 605 nm of a single IIS was generated by dividing each individual frame after the IIS (black bars) by the single frame (gray bar) that preceded the IIS (frame duration, 100 ms). C, to increase the SNR, we averaged over multiple spikes (n = 185). Each IIS crosses a certain threshold within the first 100 ms, but the timing of the peak is variable. Frame-rate and images are identical to B, in the same animal. Scale bars: 1 mm.

Fig. 17.6 ORIS of IIS at multiple wavelengths. Spike-triggered image division at A, 546 ± 10 nm (n = 134 spikes); B, 605 ± 10 nm (n = 125 spikes); C, 630 ± 10 nm (n = 75 spikes); and D, 700 ± 10 nm (n = 73 spikes) demonstrate the wavelength-dependence of the optical signal. Note that the late inverted IOS at 605 and 630 nm begins focally and then propagates in the draining veins. E, image at 546 nm shows blood vessel pattern of imaged region covered with thin cranium. Blue box is region of interest (ROI) in focus measured in F. BMI, bicuculline iontophoresis; F.P., field potential electrode. Scale bars: 1 mm. F, time course of change in light reflectance from superpixel in the focus measured at each wavelength. Scale bars: standard error of the mean (SEM).

Fig. 17.7 Ictal events imaged at four separate wavelengths in a single animal. A, f.p. recordings of four separate seizure onsets in one animal, each imaged at a different wavelength. Note the variability in the morphology of the electrophysiological trace. Horizontal scale bar: 1 second; vertical scale bars: 0.2 mV. B, images acquired every 600 ms immediately before and after the onset of the seizures shown in Figure 21.4B at each of four separate wavelengths. The denominator frame was chosen at $t = -1.2$ s, with $t = 0$ being the frame in which electrophysiological onset was observed.

Fig. 17.8 Perfusion/oxygenation events after epileptiform events. A, epileptiform events (interictal and ictal) induce a focal increase in Hbr localized to the region of active neurons (darkened pyramidal cells) because metabolism is focally increased. There is also a simultaneous focal increase in CBV. B, short or infrequent interictal epileptiform events can elicit a late BOLD signal when the increase in perfusion provides an overabundance of HbO2. C, longer events (ictal) or more frequent interictal events have a consistently high regional metabolism that outweighs the brain's ability to increase perfusion. Hence, a persistent increase in Hbr causes an inverted BOLD signal, which is also seen as an increase in reflection of light at wavelengths sensitive to hemoglobin oxygenation.

Fig. 17.9 Spatial extent of IOS as a function of wavelength. Spatial extents of the IIS were calculated by thresholding pixels to a percent above the median of the dynamic range of pixel values (12). A, spatial extents of both excitatory IOS in focus and inverted IOS in surround have a wavelength dependence. White area indicates pixels whose values are below (decrease in reflection of light) or above (increase in reflection of light) the threshold for excitatory and inverted signals, respectively. Early excitatory IOS was chosen 0.3 s after the IIS, whereas late excitatory IOS was chosen at the peak of spatial extent at each wavelength. The inverted IOS was thresholded at the maximum of the early and late peaks; in this example, these peaks occurred at 1.5 and 3 s after the IIS, respectively. Timing of images denoted by arrows in B–E. Note the focality of the IOS in the focus recorded early and the inversion of the inverted optical signal in surround comparing early with late images. Scale bars: 1 mm. Time course of spatial extent for both excitatory (closed circle) and inverted (open circle) IOS is demonstrated at B, 546 nm; C, 605 nm; D, 630 nm; and E, 700 nm.

Fig. 17.10 Illustration of proposed rings of perfusion and oxygenation surrounding an epileptic focus. An inner ring has an increase in CBV and a decrease in Hbr, whereas the outer ring has a decrease in both CBV and Hbr. The reason two separate rings are found is that the increase in CBV oversupplies the focus and spills over into the surround. This schematic is also time-dependent, because within the first 2 seconds the CBV and Hbr signals have a similar spatial extent.

Fig. 17.11 Correlation between epileptiform discharges and underlying functional architecture in ferret visual cortex. A, the timing of each IIS after initiation of movement of the grating stimuli pooled over six different animals. Each diamond represents the occurrence of an IIS in response to the stimuli displayed on the x axis. Time 0 corresponds to the onset of the movement of the orientated stimulus and the corresponding time after the blank stimulus. The y axis demonstrates the latency of the spike. Grating stimuli are more likely to elicit an IIS in a window from 40 to 300 ms after stimulus movement than are blank stimuli. B, the number of spikes that occur in a window from 40 to 300 ms

after stimulus movement in four different animals averaged over all trials. In the top two cases, oriented stimuli are equally likely to trigger an IIS. In the bottom examples, stimuli with orientations of either 0 degrees or 0 degrees and 45 degrees are more likely to trigger spikes than other orientations or blank. Error bar: SEM. Stimuli that always triggered a spike have no error bars. C, angle map generated in the presence of an IIS focus. The intrinsic signal within the focus is distorted by the occurrence of the spikes. The intrinsic signal from the surrounding cortex is unaffected. The dominant color in the focus indicates that 0° and 45° stimuli were more likely to trigger spikes than other orientations. Sample of the f.p. recording simultaneous with the imaging. Scale bar: 1 mm. From, Schwartz TH: Optical imaging of epileptiform events in visual cortex in response to patterned photic stimulation. Cereb Cortex 13:1287–1298, 2003 (63).

Fig. 17.12. The initial dip in hemoglobin oxygenation occurs early after cortical stimulation. A, surface of the brain with stimulating electrode (S.E.) under glass footplate at 546 nm. The recording electrode is outside the field of view. The yellow boxes are ROIs from which the data in B and C are taken. ROI1 sits between the stimulating electrodes and ROI2 sits over an adjacent gyrus. Scale bar: 1 cm. B, time course of intrinsic signal recorded at 546 nm from ROI1 and ROI2. Grey bar marks duration of cortical stimulation. Error bars are SD from 12 trials. C, time course of intrinsic signal recorded at 630 nm from ROI1 and ROI2. Grey bar marks duration of cortical stimulation. Error bars are SD from 12 trials. D, intrinsic signals recorded at 546 and 630 nm at varying latencies after stimulation show that although the amplitude of the signal at 546 nm is larger than at 630 nm, the initial increase in Hbr is more focal than the increase in CBV, even within the first 2 seconds after stimulation. As time passes, the CBV signal spreads diffusely throughout the cortex. At increasing latencies recorded at 630 nm, a dramatic inverse optical signal, consistent with the BOLD effect, appears in widespread cortical areas around the stimulating electrode as well as in the region of the initial dip. Scale bar: 1 cm.

Fig. 17.13 Insensitivity of IOS to electrophysiological variability. A, image acquired at 546 nm demonstrates blood vessel pattern and position of 4-AP (left) and f.p. (right) electrodes. Scale bar: 1 mm. B, f.p. recording of seizure onset. Horizontal bars are each of duration 0.6 seconds, and represent alternate camera frames. Note transition in electrophysiology after 16 seconds. C, images acquired at 605 nm corresponding to the frames indicated in Figure 21.7B. Frame 1 (not shown) was used as a denominator. Note stability of optical signal at frames 16 to 25 after the dramatic change in the electrophysiology of the seizure. Scale bar: 1 mm.