JOURNAL OF BIOMEDICAL OPTICS 1(4), 387–398 (OCTOBER 1996)

# NONINVASIVE NEAR INFRARED OPTICAL IMAGING OF HUMAN BRAIN FUNCTION WITH SUBSECOND TEMPORAL RESOLUTION

**Monica Fabiani,[†] Gabriele Gratton,[†] and Paul M. Corballis[‡]**
[†]University of Missouri, Department of Psychology, McAlester Hall, Columbia, Missouri 65211;
[‡]Columbia University, Department of Psychology, New York, New York 10027

(Paper JBO/NIR-05 received Apr. 29, 1996; revised manuscript received Aug. 20, 1996; accepted for publication Sep. 5, 1996)

## ABSTRACT

Our understanding of human brain function can clearly benefit from neurophysiological techniques capable of providing dynamic maps of activity. A series of studies is reviewed indicating that noninvasive near-infrared optical imaging methods can provide a unique combination of spatial and temporal resolution that could be used to derive dynamic maps of human brain activity. The noninvasive NIR optical data reviewed are based on the frequency-domain time-resolved measurement of photon migration parameters (intensity and delay) through brain tissue. These measurements are taken through the intact surface of the head. With these methods, two distinct components of the optical response can be identified: the ''slow optical signal'' (2–10 s latency), presumably due to hemodynamic and metabolic changes, and the ''fast optical signal'' (or event-related optical response) occurring as early as 50 to 100 ms from stimulation, and probably due to neuronal activation. *© 1996 Society of Photo-Optical Instrumentation Engineers.*

**Keywords** near infrared spectroscopy; near infrared optical imaging; event-related optical signal (EROS); photon delay; photon migration parameters; functional brain imaging.

## 1 INTRODUCTION

Neurophysiological investigations based on single-cell recordings and lesion data have emphasized that most brain functions depend on complex interactions among and within a number of brain areas.[1] The study of these interactions would be greatly facilitated by the development of methodologies that can provide different perspectives on the dynamics of the activation of various brain areas in humans. Ideally, such methodologies should be noninvasive and provide detailed spatial and temporal descriptions.

Traditional noninvasive techniques for the study of human brain function based on electrophysiological signals, such as electroencephalography (EEG), event-related brain potentials (ERPs), and magnetoencephalography (MEG), possess excellent temporal resolution (millisecond range) but variable and limited spatial resolution,[2] and are sensitive only to neurons organized in open-field configurations.[3] Recently, several imaging techniques based on changes in hemodynamics and/or metabolism, such as positron emission tomography (PET), single-photon emission computerized tomography (SPECT), and functional magnetic resonance imaging (fMRI), have provided functional maps of brain activity with high spatial resolution (a few millimeters) but relatively poor temporal resolution (on the order of seconds). Thus, each of these measures provides an incomplete view of brain activity, limiting our possibility of forming an integrated view of human cortical function. In this review we describe a new technique (noninvasive near-infrared optical imaging) that may be able to fill some of the existing gaps between electrophysiological and hemodynamic imaging methods.

### 1.1 ADVANTAGES AND LIMITATIONS OF EXISTING IMAGING TECHNIQUES

Noninvasive electrophysiological measures (EEG, ERPs, and MEG) have been used for more than 60 years in the study of brain function. The introduction of digital technology and the adoption of the averaging technique in the 1960s have greatly enhanced the temporal resolution of these measures. Specifically, ERPs can have temporal resolutions on the order of fractions of milliseconds (as in the case of brainstem evoked potentials). However, longer-latency scalp electrophysiological measures still possess relatively poor spatial resolution.[3] Although multiple dipole localization methods,[4–5] current source density mapping,[6] depth recordings,[7] and MEG[8] can, in some instances, provide tools for generating hypotheses about the intracranial generators of scalp potentials, in general

Address all correspondence to Monica Fabiani. E-mail: psymf@showme.missouri.edu

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only limited confidence can be placed in the results.[9] In addition, electrical potentials measurable at the scalp can only be generated by intracranial sources possessing an open-field geometry,[3,10] and MEG recordings are only sensitive to tangential sources possessing open-field geometry.[11]

These limitations have led investigators to search for other noninvasive methods with greater spatial resolution. Several imaging techniques (such as PET, SPECT, and fMRI) have been developed in the past decade.[12] These techniques measure hemodynamic and/or metabolic changes leading to the accumulation or change in concentration of target substances in active areas of the brain. These substances have either radioactive or magnetic properties that allow their measurement from outside the head. Functional imaging techniques can reach very high spatial resolution (on the order of a few millimeters).[2,13,14] However, their temporal resolution is usually more limited than that of electrophysiological measures. This is due to two reasons: (1) some techniques require the introduction of tracers into the bloodstream and therefore their temporal characteristics depend on the dynamics of diffusion of these substances into the brain; and (2) the metabolic and/or hemodynamic changes studied do not coincide with neuronal activity, but usually follow it by a few seconds.[15] In fact, for the measures of brain function based on hemodynamic changes, there is often a problem in interpreting the relationship between increases and decreases in blood flow and neural activity (neurovascular coupling).[16] In addition, there are often constraints in the experimental paradigms that can be used to test the subjects' cognitive activity, mostly because environmental noise levels, the presence of very high magnetic fields, or the use of ionizing radiation limit the types of stimuli that can be presented and/or the number of replications that can be obtained from a given subject.

### 1.2 OPTICAL IMAGING METHODS

A variety of optical measures have been used to study physiological parameters in humans. Their common thread is the use of visible or near-visible light. Several of these methods have been used to study brain function. By and large, two main approaches can be distinguished. One is based on the measurement of the reflectivity properties of the exposed cortex or other brain tissue, and has been used mostly in studies involving animals. The second approach is based on the measurement of near-infrared (NIR) photon migration parameters through the intact head. Because of the minimal invasivity, the latter approach can be more easily applied to studies involving human subjects. Within the photon-migration approach, two types of studies have been conducted. The first group focuses on the quantification of hemodynamic and metabolic parameters of the human brain in various physiological and clinical conditions. The second group focuses on the detection of fast changes in photon migration parameters in active brain tissue with the purpose of studying the time course of activity in localized brain areas. This paper focuses on the review of studies included in the latter group. However, since there are important relationships between these studies and those employing other approaches to optical imaging, a brief review of some of the relevant work done with other methods is also provided.

## 2 OPTICAL IMAGING OF THE EXPOSED CORTEX

Optical imaging methods have been applied to the exposed cortex of animals (such as cats or macaque monkeys) and have produced maps of brain activity with high spatial and temporal resolution.[17] Specifically, most optical studies of the functional architecture of the cortex have involved measurements of the reflectivity of the exposed cortex of animals,[18] and, in some cases, of humans during surgical operations.[19–20]

Recently, it has been demonstrated that reflectivity measures can also be taken through the dura and thinned skull,[15] or from the hippocampus of freely moving animals.[21] These studies have shown that reflectivity measures of the exposed cortex are very sensitive, and possess high spatial resolution (50 $\mu$m). Their temporal resolution varies, depending on the type of signal observed. Researchers in this area have emphasized a distinction between an intrinsic signal (arising from changes in cortex reflectivity under stimulation) and an extrinsic signal (which is studied by using voltage-sensitive dyes).[18] The intrinsic signal is usually considered to be relatively slow (taking a few seconds to reach its maximum) with respect to the extrinsic signal (which can reach a temporal resolution on the order of milliseconds). This difference is attributed to the fact that the intrinsic signal is due to metabolic and hemodynamic changes while the extrinsic signal directly reflects the electrical activity produced by the neurons.

Recent observations[22] (see also Frostig et al.[15]), conducted with a methodology combining continuous spectroscopic methods with high temporal resolution, have shown that the intrinsic optical signal elicited by sustained visual stimulation in the occipital cortex consists of at least three different signals. The first of these signals is due to scattering effects. It is highly localized and its time course is similar to that of neuronal activity. This signal is most evident in the near-infrared range, where the absorption of hemoglobin is relatively low with respect to the visible range.

The second signal is an increase in the concentration of deoxyhemoglobin, which begins within 500 ms from stimulation and declines shortly after the end of the stimulation period. This signal is also

very well localized to the active brain areas and suggests an increase in aerobic metabolism in these regions.

The third signal is due to an increase in the concentration of oxyhemoglobin, and is relatively slow both in its onset (a few seconds from stimulation) and in its decay. This signal is also less well localized than the other two signals and can be observed at a distance of millimeters from the active brain areas. This signal presumably corresponds, at least in part, to the blood-oxygenation-level dependent (BOLD) oxygenation signal observed with fMRI and may also be related to the increased blood flow observed with PET. Therefore, the data reported by Malonek and Grinvald[22] suggest that optical methods may provide images of brain activity with a very good temporal resolution, in particular if they focus on measures of light scattering. Furthermore, their data indicate that changes in light scattering are responsible for most of the intrinsic signal observed using near infrared light. In the remainder of this paper we focus on optical measures based on near infrared light, conducted noninvasively in humans.

## 3 NONINVASIVE OPTICAL IMAGING OF BRAIN FUNCTION IN HUMANS

Among the various methods proposed for deriving functional images combining good temporal and spatial resolution are photon migration techniques.[23–24] Photon migration methods are based on the measurement of parameters of the migration of near infrared photons traveling through the tissue. These parameters are likely to depend on the scattering and absorption properties of the medium. Two types of techniques are used. One of them is based on applying a continuous light source to the surface of the body, and measuring the amount of light that reaches a detector, located at some distance (for the purposes of this paper, we label this parameter ''intensity''). Using light sources of different wavelengths, it is possible to derive intensity spectra that can be used to estimate the concentration of various functionally significant substances within the body (see for example, Wyatt et al.[25]). This approach is very economical, and commercial systems are available. A problem with this approach is that the measurements will be influenced not only by absorption, but also by scattering changes, and it is sometimes difficult to separate the relative contributions of these two factors. Another problem with this approach is its limited spatial resolution: since body tissues are for the most part highly scattering, the photons diffuse widely through the medium and the volume explored by the measures is very large.

A second approach (time-resolved optical methods) takes into account not only the probability of photons emitted by the source reaching the detector, but also the time they take in their migration through the tissue—''time of flight,'' or ''delay.'' Patterson et al.[26] showed that measurement of the distribution of the photon delay makes it possible to quantify separately the scattering and absorption coefficients of tissue. A further advantage to time-resolved methods is that they can be used to derive measures with a good spatial resolution even in the case of scattering media.[24] This is because time-resolved methods allow the measures of photon migration parameters to be based on only those photons that travel relatively fast through the medium. These photons are likely to remain within a very circumscribed volume between the source and the detector. The width of this volume depends on the criterion used to select the photons (the faster the selected photons, the narrower the volume, and the higher the spatial resolution).

The measurement of photon migration parameters is a completely noninvasive procedure. The wavelength of the light (NIR) does not pose any special danger, and the intensity of the sources (on the order of microwatts) guarantees that the amount of energy involved does not pose special risks and is below the subject's sensory threshold. The use of light-emitting diodes (LEDs) makes the procedure particularly safe, since these sources can be handled without the need for special precautions.

Consideration of the optical properties of the various anatomical structures of the head and the results of several published studies indicate that it is possible to apply optical measures to the noninvasive study of human functional brain activity. In the remainder of this section we first present biophysical and physiological considerations that are the basis of photon migration methods, and then describe two types of optical signal and some methodological aspects of the noninvasive recording of brain activity.

### 3.1 BIOPHYSICAL BASES OF PHOTON MIGRATION METHODS

Noninvasive measurement of the optical properties of areas of the body requires a source emitting light of a particular wavelength (in the NIR range) that illuminates a point on the surface of the skin, and a detector, also located on the skin at some distance from the source. In the case of NIR light, living tissue behaves as a scattering medium. This means that light propagation can be described as a diffusion process.[27] In the absence of absorbing structures, the light intensity and the distribution of the photons' time of flight are a function of the source-detector distance and of the scattering coefficient of the tissue itself.

The scattering coefficient $\mu_s$ of living tissue is on the order of about $10^3$ to $10^4$ $mm^{-1}$ and is essentially due to forward scattering.[28–32] Measurement of the scattering coefficient is difficult. However, for practical purposes it is sufficient to consider the reduced

scattering coefficient $\mu_s=(1-g)\mu_s$, which takes into account the average of the cosine of the scattering angle. The reduced scattering coefficient is still on the order of 1 to 10 $mm^{-1}$. In addition, most parts of the body also absorb light to some degree and, in turn, this influences light intensity and, indirectly, the average photon delay. Typical values of the absorption coefficient $\mu_a$ in animal tissues are on the order of 0.01 $mm^{-1}$.[29] Given the typical scattering and absorption coefficients of living tissue, the Boltzmann's transport equation for photons can be solved in the diffusion approximation.[33]

Several researchers have experimentally demonstrated the validity of the diffusion approximation in typical tissue.[28,34–37] This provides the physical arguments necessary for the estimation of the scattering and absorption coefficients. Indeed, Patterson et al.[26] showed that intensity and delay parameters of near infrared photons injected into living tissue can be used, at least in some cases, to estimate the absorption and scattering coefficients of the tissue itself.

### 3.2 TIME-RESOLVED MEASUREMENT OF OPTICAL PARAMETERS

The first instances of photon migration techniques, based on the estimation of delay parameters in the time domain, were too slow for the study of the neuronal dynamics associated with brain function.[38] E. Gratton et al.[39] developed a frequency domain method to assess the parameters of the statistical distributions of intensity and time of flight. With this technique, a sinusoidally modulated light source is used to launch a photon density wave. If the medium is uniform and infinite, the photon density wave travels at a constant velocity and is exponentially attenuated as it propagates.[35] The attenuation values and the phase delay of the wave are a function of both the scattering and the absorption coefficients of the medium. In a uniform medium, simultaneous measurements of the phase delay and of the modulation of the photon density wave are sufficient to uniquely determine the values of the absorption and scattering coefficients. In the frequency domain, the presence of absorbers or scatterers results in a deformation of the photon density wave. This deformation is equivalent to the diffraction of waves by obstacles. The photon density wave exhibits wave phenomena, such as reflection and refraction at surfaces with different wave velocity. A distinct advantage of frequency domain methods for the study of brain activity is that the values of the wave's phase and modulation can be easily measured and continuously monitored using relatively simple detectors and electronics.

The frequency domain methods used in photon migration studies derive from instruments developed for fluorescence decay kinetics.[40–41] They consist of a light source modulated in the megahertz or gigahertz range and of a heterodyned detector. Both single pixel detectors and array detectors have been used to study photon migration. The heart of a frequency domain spectrometer is a set of coupled frequency synthesizers that provides two frequencies in the 100-MHz range which differ from one another by up to 10 kHz. The difference between the two frequency synthesizers is used to modulate the light source. In our case, the synthesizer drives a radio frequency amplifier modulating the current in the laser diode. The second synthesizer is used to modulate the gain of the dynodes of the photomultiplier amplification chain. Effective gain modulation of about 100% can be obtained. The gain modulation produces a shift of the high-frequency signal detected by the photomultiplier to a convenient low frequency, at which accurate digital filtering methods can be applied. The latter is performed through a high-speed *A-D* converter board. The final result is an accuracy of the phase measurement of about 0.1 degrees (out of 360 degrees) and of the modulation determination of about 0.1%. This kind of accuracy is necessary for measuring the delay and attenuation of the photon density wave front in response to small variations (on the order of a few picoseconds) in the activity of the brain. In all experiments performed by G. Gratton and colleagues and summarized in Sec. 5 of this paper, the entire measurement is under computer control.

### 3.3 PHYSIOLOGICAL MECHANISMS UNDERLYING PHOTON MIGRATION EFFECTS

As mentioned, the parameters of photon migration are influenced by the scattering and absorption properties of the tissue, which, in turn, are differentially affected by such factors as the shape and/or reflectivity of cell membranes, concentration of metabolically significant substances (such as oxy- and deoxyhemoglobin, and cytocromes), size of the blood vessels, etc.[17,22] Various types of metabolic and functional phenomena are likely to influence the optical properties of tissues. For example, action potentials produce changes in the scattering coefficient, presumably because of the movement of ions.[42] Stepnoski and colleagues[43] have suggested that rapid reorientation of membrane dipoles contributes to activity-dependent changes in light scattering in *Aplysia* neurons. Changes in scattering associated with neuronal activation have also been demonstrated in bloodless hippocampal and cortical slices, as well as in the isolated brain.[15,44–45] In addition, both scattering and absorption of NIR light may be influenced by changes in the concentration of metabolically significant substances.[23]

As mentioned earlier, *in vivo* changes in optical properties have often been measured using light reflected from the exposed cortex (intrinsic signal).[17,18,22] However, there are several fundamental differences between measurements of intrinsic cortical light reflection and photon migration (or

transmission) measures: (1) transmission measures are taken noninvasively in a behaving human subject (i.e., through the skin, skull, and meninges); (2) transmission measures are based on parameters of the migration of photons through tissue (attenuation and delay of the light between the source and the detector); and (3) transmission studies have focused on wavelengths in the near infrared range because of their high penetration, whereas reflectivity studies usually use light in the visible range. For these reasons, intrinsic reflectivity measures are likely to be dominated by changes in tissue coloration determined by changes in blood flow or oxygenation level, whereas transmission measures (and in particular measures of photon delay) may be less affected by these factors and influenced more by changes in scattering (such as those that have been described to be associated with neuronal activity).[15,22,37–39] Although it is unlikely that noninvasive photon migration measures in humans will reach the same spatial resolution as reflectivity measures obtained from the exposed cortex, they should provide deeper penetration, and, of course, be more widely applicable.

### 3.4 FAST AND SLOW COMPONENTS OF THE OPTICAL SIGNAL IN RESPONSE TO STIMULATION

As mentioned, several observations provide evidence that noninvasive optical imaging of deep structures is possible[24,46] and that optical methods are sensitive to functional changes of brain activity.[47–50] Specifically, G. Gratton and colleagues[47] identified two types of functional effects: Fast effects (with a 50 to 500 ms latency from stimulation) and slow effects (with a 2 to 10 s latency from stimulation). Monte Carlo simulations indicated that fast effects are consistent with changes in the optical properties of relatively deep layers of the head (such as the cortex), while the slow signals are consistent with changes in deep and/or superficial layers. The properties of the fast optical signal are also consistent with the hypothesis that they may reflect neuronal activation, whereas those of the slow signals are consistent with the hypothesis that they correspond to hemodynamic and/or metabolic changes.

In the experiments conducted by G. Gratton et al.,[47–48] optical parameters (photon intensity and delay) were recorded continuously over trials or short blocks of a duration of approximately 20 s. A short (6 or 10 s) interval at the beginning of the block was free of stimulation (baseline period), and was followed by a stimulation period. During the stimulation period, several stimuli were presented at fixed intervals. A short rest period was given in between trials or short blocks. Finally, trials or short blocks were repeated a number of times for each subject, condition, and recording location. This procedure allowed for the observation of systematic

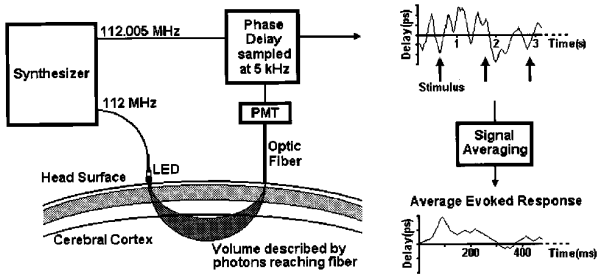


**Fig. 1** Schematic representation of the apparatus used for recording optical parameters (left) and of the methods used for deriving an average evoked optical response (fast optical signal, right).

shifts in optical parameters, which occurred over the entire stimulation period (slow optical signal). G. Gratton et al.[46] assumed that relatively slow hemodynamic and metabolic changes were the most important cause of these slow drifts (see also Frostig[17] and Malonek and Grinvald[22]). These slow changes may also correspond to the activation effects observed with other functional imaging techniques.[2,12]

Fast optical signals were obtained by analyzing the optical response time locked to the presentation of individual stimuli during the stimulation period (see Figure 1). The stimulation period was segmented into 500 ms epochs, each time locked to the presentation of a stimulus. Then the optical parameters obtained in epochs corresponding to the presentation of the same stimulus were averaged together for each subject, condition, and scalp location. This procedure (which is analogous to the averaging usually performed with event-related electrical brain activity) allows for the identification of the optical response immediately following the stimulation. Because of the short latency of these signals, neuronal activation is a likely candidate for the basic mechanism of fast optical measures.

### 3.5 RECORDING OF EVENT-RELATED OPTICAL RESPONSES AND POSSIBLE SOURCES OF ARTIFACT

In this section we review some of the issues relating to recording artifacts and other limitations encountered in the recording of event-related optical signals. Similar problems apply to both fast and slow optical signals.

Given the small amount of light used for brain measurements, the presence of hair (especially if dark colored) poses a problem. This problem can be addressed by combing the hair away from the optic fiber, so that there can be close coupling between the optic recording devices and the skin. Skin color does not appear to pose particular problems.

There may also be recording artifacts (or diminished coupling) due to movements of the recording device with respect to the head. Those are usually characterized by a minimum of 10% change in the

intensity reading from one trial to the next. Trials in which these artifacts occur are discarded.

The pulsation of the vascular system associated with systolic activity of the heart produces relatively large changes in the transmission of light through tissue.[51] This may produce substantial artifacts that are difficult to eliminate with simple filtering approaches. Note, however, that although the pulsation signal can obscure fast optical effects and should therefore be removed before analyzing the fast optical signal, it can be an important effect when the focus is on hemodynamic phenomena. G. Gratton and Corballis[52] developed an algorithm to compensate for this artifact based on a regression procedure in which the effect of systolic pulsation is estimated from the data. The procedure takes into account beat-to-beat variability in pulse rate and amplitude. Tests showed that this procedure substantially reduces the impact of the pulsation artifact. This procedure is applied to all the data that are described in the following section.

Most of the functional noninvasive optical research conducted so far has been performed using a 1-channel optical recording system. Therefore, data from different placements over the head need to be recorded in separate experimental sessions (although some locations were recorded twice for reliability testing). This problem could be overcome by using a system that allows for parallel recordings from multiple locations. Such a system is under development.

## 4 STUDIES OF EVENT-RELATED OPTICAL RESPONSE (FAST OPTICAL SIGNAL)

In this section, we review research conducted during the last few years. The main finding is that photon migration measures appear to be able to provide direct, noninvasive indices of neuronal activity which, in turn, can be used to derive functional maps of cortical activation with good spatial and temporal resolution.

### 4.1 PATH OF INFRARED LIGHT IN SURFACE-BOUND SCATTERING MEDIA

These initial experiments were conducted on phantoms.[46,53] Their purpose was to acquire information about the path traveled by photons between a source and a detector located on the same surface. This information is critical for using photon migration parameters to assess regional changes in brain activity, since only absorbers and scatterers located along this path will influence the measures. Light produced by a laser was projected on the surface of a tank filled with a scattering suspension representing a first level of approximation to the conditions observed in the human body. These studies showed that the presence of a surface boundary with a nonscattering medium (such as air) produces a distortion of the average light path between the source and the detector. The resulting path is curvilinear, that is, photons travel deeply into the medium before reaching the surface again (see left panel of Figure 1). The average depth reached is slightly more than half the distance between the source and the detector. Given the stochastic nature of the diffusion process, the cross-section of the path is best described as a distribution of probability densities, which varies as a function of the distance from the source and from the detector. The parameters of this distribution depend on the parameters of the light used. The width of the distribution is greater for light of fixed intensity than for frequency-modulated light, and higher modulation frequencies correspond to narrower distributions. This is of practical importance because narrower path distributions may provide better spatial resolution. In the study by G. Gratton et al.,[46] the effects of bone tissue on the ability to detect intracranial absorbing objects using optical measures were also evaluated. A sheep skull was submerged in the scattering suspension and an absorbing sphere of 8 mm diameter was placed inside the skull. The effect of the sphere was demonstrated by measuring changes in photon migration parameters at the surface of the suspension with and without the sphere inside the skull.

The data obtained by G. Gratton et al. confirmed earlier observations that NIR light can penetrate through the skull, so that optical properties of brain structures can be studied noninvasively, at least at near-infrared wavelengths. However, some of these earlier observations[54] were obtained on newborn infants, whose skulls may be much less calcified than those of adults.

Although these data support the claim that noninvasive optical imaging is possible, it should be noted that the optical properties of various brain structures are far from homogeneous. For instance, the scattering coefficient of the white matter is higher than that of the gray matter.[55] For this reason, near-infrared photons traveling through the brain are likely to remain confined to the gray matter, rather than penetrate the white matter (a similar phenomenon may also occur at the interface with the skull). Thus, surface measures of brain optical properties are likely to be biased in favor of effects localized in the gray matter rather than of those localized in the white matter. This may actually magnify the signal, although it may also introduce irregularities in the surface maps.

### 4.2 DETECTION OF FUNCTIONAL CHANGES IN BRAIN ACTIVITY

The purpose of the studies summarized in this section was to show that photon migration methods can detect localized changes of brain optical parameters directly associated with neuronal activity. Several investigators have reported changes in cerebral optical properties during the performance of sensory, cognitive, or motor tasks.[45,46,56,57] These studies employed steady-state optical methods, and, for

the most part, focused on changes in the concentration of oxy- and deoxyhemoglobin associated with brain activity. These changes were quantified using light of different wavelengths, and were based on the characteristic absorption spectra for oxy- and deoxyhemoglobin (*in vivo* spectroscopy). For instance, Kato et al.[49] studied changes in hemoglobin concentration in occipital areas during a photic stimulation task. They reported an increase in oxyhemoglobin after simulation, followed by a slower and less pronounced increase in deoxyhemoglobin. The time course of the effect was, however, quite slow. Kato et al. used a sampling rate of 5 *s*, and reported changes occurring over several minutes of stimulation. Similar results were reported by Obrig et al.[49] from the precentral cortex during a unimanual tapping task. In addition, Obrig et al.[50] showed a relationship between these effects and measures of blood flow changes obtained with other imaging techniques. Hoshi and Tamura[57] employed a similar approach to study changes during the performance of more complex tasks requiring subjects to answer various types of questions. All of these data provide quantification of oxy- and deoxyhemoglobin cerebral concentrations; however, given the use of a steady-state recording system and a relatively slow acquisition rate, these data provide limited spatial and temporal information.

Recordings of fast changes in brain optical parameters were first made by Chance et al.[56] In this study, subjects were required to answer complex questions. During this task, optical parameters were recorded using a steady-state system placed on the forehead. The results were analyzed in terms of frequency spectra during the task condition compared with similar spectra obtained during a rest period. Using this approach, Chance et al. reported the observation of rhythmic oscillations in the transparency of frontal cerebral areas to NIR light, with frequencies of 1–4 Hz. The exact nature of these changes remains unclear. It should be noted, however, that the use of steady-state recording does not allow for good spatial localization of the signal.

In order to address some of these problems, G. Gratton and colleagues used fast-paced (more than 10 Hz) recording from multiple sites employing time-resolved frequency domain methods. In addition, they also used tasks with discrete stimuli and/or responses in order to be able to correlate more directly the stimulus presentation with the brain response. This allowed for the observation of fast, localized, functional brain activity. Finally, as mentioned above, procedures for dealing with artifacts such as those related to the pulsation of the vascular system were used in all studies.[52]

#### 4.2.1 Tapping Task

G. Gratton et al.[47] recorded optical parameters from motor areas of the brain during the performance of a rhythmic motor task. Among the conditions in this experiment was one in which subjects had to tap with their right or left hand. Photon migration parameters obtained from probes located to the left and right of the midline (close to the vertex) were compared. In these conditions, the path of light was expected to cross motor areas of either the left or right hemisphere, depending on the location of the probe. In each case it was expected that, if photon migration parameters are sensitive to functionally significant changes of regional brain activity, they would show differences between recordings obtained when the tapping was performed with the hand contralateral or ipsilateral to the probe. Parameters of the light reaching a detector were derived in two conditions: with the light source located either 3 cm to the left or to the right of the vertex (at which the detector was located). Four subjects were asked to tap for 20 s at a frequency of 0.8 Hz. Ten seconds of rest preceded and followed the tapping period. Systematic differences emerged as a function of movement side (i.e., contralateral or ipsilateral to the light source), which are consistent with the known functional neuroanatomy of the motor cortex. In particular, both slow and fast effects were described. Slow effects were made evident by the comparison of the average photon migration parameters during the tapping and rest periods. Fast effects were changes in the optical parameters of the brain that occurred at the tapping frequency (0.8 Hz) and/or its harmonics.

The critical comparison in this study was the difference in the optical signal while tapping with the contralateral and ipsilateral hand. Statistical analyses revealed the presence of contralateral activity for the delay parameter. In order to provide a framework for interpreting these results, a series of Monte Carlo simulations were run which investigated the effects of changes of scattering and absorption in layers (simulating the skin, skull, meninges, cortex, etc.) located at various depths in a semi-infinite, homogeneous scattering medium (simulating the head) on the photon migration parameters. The results of these simulations suggested that slow effects are consistent with changes in either superficial or deep layers, whereas fast effects are only consistent with changes in deeper layers (at least 1 cm deep). These results provide support for the claim that surface measures of optical parameters can detect functional changes in the scattering (or absorption) properties of deep structures, such as the cortex. Some of the changes have a frequency of at least 0.8 Hz, and can discriminate between contralateral and ipsilateral activity in the motor cortex.

#### 4.2.2 Quadrant Stimulation Study

The spatiotemporal characterization of the optical signal derived from the tapping experiment was still very crude. A visual stimulation study was run to provide better estimates of the time course and degree of localization of the optical signal.[48] Specifi-

cally, G. Gratton et al. wanted to determine whether fast optical signals could be used to derive a retinotopic map of the visual cortex. To gain more knowledge about the time course of the fast optical signal, a faster sampling rate (20 Hz) than previously used was employed to record time-locked optical cortical responses. Four black-and-white vertical grids were displayed in the four quadrants of a computer monitor. In each trial, one of the grids switched colors every 500 ms. The alternating grid was varied across trials. This was expected to produce systematic variations of the segment of primary visual cortex stimulated in each trial.

Twelve trials were run for each of the four quadrant-stimulation conditions. Photon migration parameters (light intensity and delay) were measured from 12 scalp locations over the occipital lobe. Recordings corresponding to 456 reversals for each stimulation condition were obtained for each location. These data were used to derive average waveforms corresponding to the changes in optical parameters during the first 500 ms after stimulation evoked by reversals of the different grids. The most important feature in the average waveforms was a deflection peaking 100 ms after stimulation for the delay parameter and reaching a maximum delay of about 10 ps (1 ps=$10^{-12}$ s, see Figure 2). Little or no effect was observed for the intensity parameter. For each stimulation grid, the recording location with the maximum peak response was identified.

The maps (reported by G. Gratton et al.,[48] and reproduced in Figure 3) indicated that the areas of maximum response for each of the four stimulation conditions were located according to the expected retinotopic map of the visual field in the primary visual cortex (i.e., the maps were inverted along both the vertical and horizontal axes). The analysis was carried out in the following way: (1) peaks of activity for each subject and experimental condition were identified; (2) the horizontal and vertical coordinates of these peaks were subjected to separate ANOVAs (using left-versus-right quadrant stimulation and top-versus-bottom quadrant stimulation as factors). The ANOVA revealed main effects of both left-versus-right quadrant stimulation on the horizontal coordinate of the peaks, and upper-versus-lower quadrant stimulation on the vertical coordinate of the peaks. The separation between the left and right loci of maximum response (1.5–3 cm) was consistent with fMRI data,[58] although the temporal resolution of the optical data was much greater than that obtained with fMRI. The separation between the loci of maximum response for top and bottom visual field stimulation (0.5–1 cm) was consistent with the known neuroanatomy of the striate cortex (area 17), in which these areas of the visual field are mapped onto the superior and inferior borders of the calcarine fissure.

Although direct data on the depth of the effects are not available, the simulations reported by G. Gratton et al.[47] suggest that changes of about 10 ps in the delay parameter combined with virtually no change in the intensity parameter are consistent with variations in scattering (or absorption) of layers 1.5–3 cm deep (approximately the depth of the superficial half of the calcarine fissure). Note that the latency of the effects makes it unlikely that they reflect hemodynamic changes. Rather, these changes appear more consistent with the scattering changes that are supposed to coincide with electrical neuronal activity, as proposed by Stepnoski et al.[43] (see also Frostig[17]). These effects are presumably due to changes in the shape and reflectivity of neurons associated with membrane polarization and depolarization, synaptic activity, etc. The latency and spatial characteristics of the observed data are therefore consistent with the hypothesis

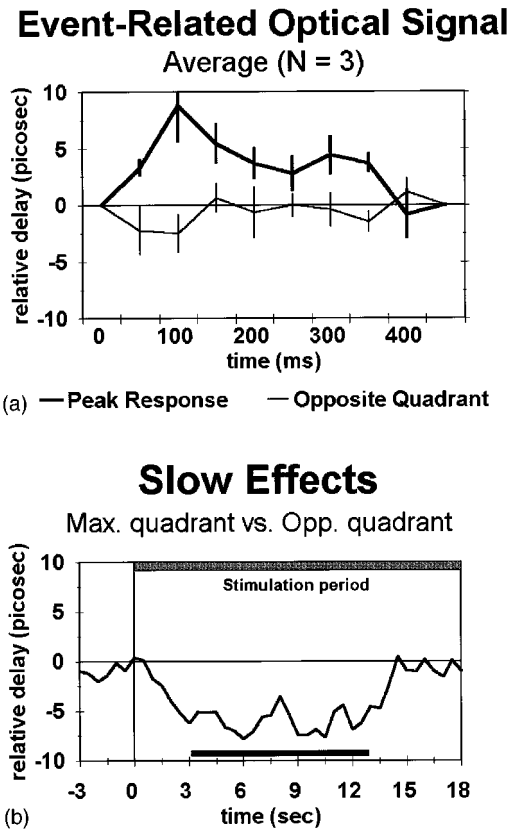


**Fig. 2** (a) Grand average delay versus time functions at the location of maximum response. The data obtained from locations of maximum response for a particular stimulating quadrant are compared with those obtained at the same locations when the opposite quadrant was stimulated. The data were averaged across stimulation conditions. The error bars refer to the standard error computed across subjects. (b) Changes in delay over the stimulation period. Data are presented as differences between the values of delay for locations of maximum response for a particular stimulating quadrant (identified as above) and the values of delay recorded at the same locations during stimulation of the opposite quadrant. Data are accrued over periods of 0.5 s. The gray bar indicates the period of stimulation. The dark bar indicates the period in which the difference between the responses to stimulation of the two quadrants was significant, $t(2)=4.90$, $p<.05$. (© 1995 by the Society for Psychophysiological Research. Reprinted with the permission of Cambridge University Press.)

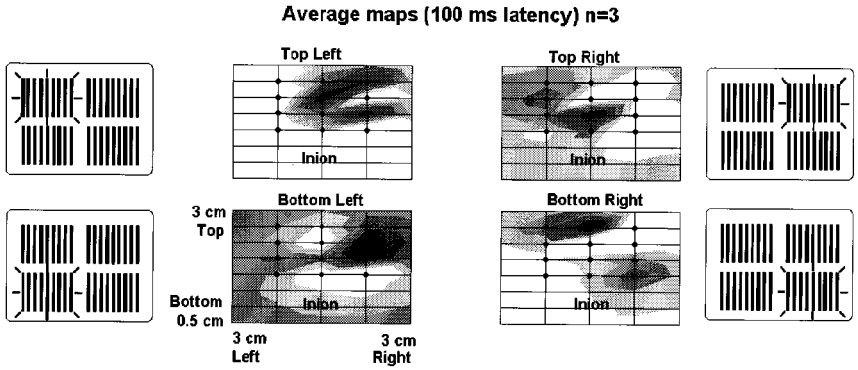


**Fig. 3** Average surface maps of changes in the delay parameters with respect to a prestimulus baseline over occipital areas ($N=3$). The maps are derived from range-corrected data (i.e., the gray shadings indicate relative values). Areas with the largest relative increments in the photons' delay (with respect to prestimulus values based on a 100 ms period preceding the grid reversals) are indicated in black, and those with the largest relative decrements are indicated in white. The locations of the twelve measurement points are indicated by filled circles. The coordinates are expressed as distance from the inion in centimeters. The diagram to the side of each map indicates the stimulation condition corresponding to that map. (© 1995 by the Society for Psychophysiological Research. Reprinted with the permission of Cambridge University Press.)

that photon migration parameters can be used to monitor in a quasi-continuous, noninvasive manner neuronal activation in cortical areas (in this case, the striate cortex). These observations suggest that the temporal resolution of the technique may be at least as high as 50–100 ms, and its spatial resolution on the order of 5 mm.

### 4.2.3 Pilot Studies on Visual Attention and Color Processing

This section reviews two studies that are currently in progress. The purpose of the first study is to evaluate the ability of optical methods to separate functionally adjacent brain areas. The experiment is based on a visual-spatial attention paradigm. Previous research[59] has suggested that the primary visual cortex responds similarly to stimuli presented in attended and unattended locations, while a differentiation in the response to these types of stimuli can be observed further along the visual pathway. If this is true, and if noninvasive optical methods can be used dissociate functionally adjacent brain areas, then we should observe ''attention effects'' (i.e., larger response to attended than to unattended stimuli) when optical measures are taken from lateral occipital areas but not from medial areas. The medial areas, on the other hand, should respond equally to attended and unattended stimuli, and therefore show a ''stimulation effect.''

In the present experiment, optical data were recorded from an area of the scalp covering both striate and portions of extrastriate cortex. The paradigm adopted was similar to that employed by Mangun and Hillyard.[59] In particular, during different blocks, the subject's attention was directed either to the left or to the right of a fixation cross. The stimuli were squares or (rarely) rectangles that were randomly flashed for 50 ms on each side of the cross. The subject was instructed to respond by pressing a button when a rectangle was presented on the attended side. Optical recordings were obtained at 50 Hz from 28 locations covering an area 1.5 cm high and 7.2 cm wide, centered 2.25 cm above the inion.

The data were analyzed by computing separate averages for attended and unattended stimuli presented to the left and right of fixation (separately for each subject and location). The average of the optical responses for attended and unattended stimuli was labeled the ''stimulation effect,'' while the difference between these responses was labeled the ''attention effect.'' Separate maps for stimulation and attention effects were then constructed for each subject. They indicated that stimulation effects were evident at medial locations, whereas attention effects were evident at more lateralized locations. The time course of the optical activity for attended and unattended stimuli suggested that little difference could be found between the responses to attended and unattended stimuli at medial areas. However, a clear difference was found at lateral areas. In fact, at lateral locations, a reduction in photon delay was observed after the presentation of unattended stimuli. The functional significance of this phenomenon remains to be specified, although it may suggest the existence of inhibitory processes. However, the results are consistent with the hypothesis that attention effects are visible in extrastriate but not in striate cortex. In addition, these data provide a demonstration of how optical data can be used to compare the activity of adjacent cortical areas.

Although it is possible to postulate the existence of a correlation between optical and electrical responses obtained in similar paradigms, it is also likely that optical and electrical data could be differentially influenced by different types of neuronal activity. For instance, surface electrical data only reflect the activity of neurons organized in open-field configurations—such as pyramidal cortical neurons.[3] This type of geometric limitation is not likely to influence the optical measures (which are mediated by a random diffusion process). Therefore, it is likely that optical measures may also be influenced by neurons organized in closed-field configurations—such as stellate cortical neurons.

An example of dissociation between electrical and optical data was obtained in a study (still in progress) on the evoked response elicited by patches of different color. Subjects were habituated to a yellow background and red or blue patches were briefly flashed over this background. On the basis of existing knowledge on color vision pathways,[60] it was hypothesized that the red and blue stimuli would elicit different brain responses, and, in particular, that the latency of visual evoked potentials (VEPs) and optical responses to blue patches would be longer than that to red patches. VEPs were recorded at 200 Hz from 22 locations over the posterior scalp. Optical responses were recorded at 50 Hz from 28 locations over the occipital region (in different sessions). As expected, for both optical and electrical measures, the latency of the earliest evoked response was longer for blue than for red stimuli. In addition, while the blue patches elicited an optical response with a latency of approximately 80 ms, scalp electrical activity in this condition was not evident until 200 ms after stimulation. This was true for all 22 electrodes used in the experiment, indicating that the electric field over the back of the head was approximately constant until 200 ms after stimulation. This strongly suggests that no detectable open-field activity was generated during this interval. However, an optical response was clearly visible during the same period. This indicates that an optical response can be recorded in the absence of a surface electrical potential. One explanation for this discrepancy is that the optical activity observed was generated by neurons organized in a closed-field configuration. Another explanation is that the activity was generated by neurons facing each other, such as the neurons located on opposite banks of the calcarine fissure. The electric fields generated by these neurons would tend to cancel each other, but such a cancellation should not occur for the optical measures. Regardless of the explanation, these data clearly point to the ''uniqueness'' of the information provided by the optical data, and to the increased analytical power obtained by combining optical and electrical measures.

## 5 SUMMARY AND DISCUSSION

Taken together, these experiments suggest that photon migration techniques possess the depth of penetration, the temporal and spatial resolution, and the sensitivity required for noninvasive measurement of regional brain activity in human subjects. The data obtained so far also suggest that optical methods can provide information that complements and integrates that obtained with other techniques, and that a combination of optical, electrophysiological, and hemodynamic methods may be particularly fruitful.

On the basis of the fast signal, maps demonstrating a spatial resolution of at least 5 mm were obtained. In further studies using visual stimulation, we also obtained evidence that (1) optical activity can distinguish between stimulation effects (localized to area 17) and attention effects (which are evident in extrastriate areas of the occipital cortex); and (2) in some cases the optical cortical responses may precede or occur in the absence of surface electrical activity (suggesting the presence of closed-field neuronal activity).

These data suggest that noninvasive optical imaging possesses the desired characteristics for filling some of the gaps left by electrophysiological and imaging data. Specifically, a possible approach to the problem of describing cortical activity in humans is a combination of techniques with high temporal resolution (such as electrophysiological methods) and spatial resolution (such as hemodynamic techniques). However, this integration is made difficult by the large gaps at present existing between methodologies that achieve maximum resolution in the temporal and spatial domains, respectively. These gaps derive not only from the different types of information provided, but also from differential sensitivity to various types of brain activity. For instance, electrical measures are sensitive only to open-field activity, while hemodynamic measures may require the sustained activation of brain areas. It is possible that using optical methods in combination with electrophysiological recordings and fMRI will help fill the gaps existing between currently available methodologies.

The approach used in the Gratton et al. work reviewed in this paper is purely functional and no attempt was made to quantify absorption and scattering coefficients. This was due, in part, to technical limitations such as the use of a single wavelength. However, other laboratories have successfully used photon migration methods to derive precise spectroscopic measurements of the concentration of oxy- and deoxyhemoglobin in the brain, and reported changes during the performance of various tasks.[16,24,54,57] Benaron and colleagues have recently reported functional quantitative images of brain activity derived in this fashion. A combination of a quantitative approach with the use of fast measurements of brain activity may be

very important for the future development of noninvasive optical imaging of brain function.

In future research, it will be important to address the following issues:

1. Advancement in the understanding of the basic mechanisms involved in the fast and slow optical signals. An initial approach to this problem is to determine whether these effects are due to absorption and/or scattering changes in tissue as well as to explore their relationship with the reflectivity data obtained from the exposed cortex.

2. Evaluation of the depth information provided by optical data (especially with respect to the issue of 3-D reconstruction).

Thus, in conclusion, although noninvasive optical methods are still evolving, their unique combination of spatial and temporal resolution appears very promising in the study of the dynamics of human brain function.

### Acknowledgments

The work described in this review paper was supported in part by National Institutes of Health grant R01MH 54110-01A2 to Gabriele Gratton.

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