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Noninvasive Detection of Fast Signals from the Cortex Using Frequency-Domain Optical Methods

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INTRODUCTION

The study of the functioning of the human brain has recently become one of the most significant areas in science, as demonstrated by the official declaration of the 1990s as the "Decade of the Brain." A considerable amount of research has focused on the study of physiological phenomena associated with sensory, cognitive, and motor functions. Theories in these areas emphasize that these functions depend on the dynamic interaction of different brain areas.[1,2]

The last few years have also seen a considerable expansion in the physiological methods used to examine the functioning of the human brain. By and large, two major classes of techniques have evolved: techniques measuring the electromagnetic fields produced by active neurons (such as event-related potentials—ERPs—and magnetoencephalography—MEG), and techniques measuring hemodynamic or metabolic changes that are associated with neuronal activity (such as positron emission tomography—PET—and functional magnetic resonance imaging—fMRI). The types of information that are derived from these two classes of techniques are basically different. Electrophysiological techniques provide a direct, on-line window on the functioning of the brain, with excellent temporal resolution. Hemodynamic methods provide summaries of changes in metabolic rate in different brain areas during the performance of a task, and possess good to excellent spatial resolution.[3] Although the information provided by each of these approaches is invaluable, the study of the interactions between brain areas would clearly benefit from a method able to integrate temporal and spatial information, so that it would be possible to determine the relative timing of activation of

specific brain areas. In this paper we will review recent data suggesting that noninvasive optical imaging may have the desired temporal and spatial resolution to provide this type of information and may help integrating electrophysiological and hemodynamic measures of brain function.

FUNCTIONAL CHANGES IN OPTICAL PROPERTIES OF NEURAL TISSUE

It has been known for some time that the optical properties of the cortex change when the cortex is functionally active. This has been demonstrated by measuring the reflective properties of the exposed cortex of animals.[4] Using this approach, it is possible to derive maps of functional cortical architecture that have a spatial resolution of up to 50 μm.[5] These studies have suggested that both absorption and scattering properties of neural tissue may change with activation.[6–8] Absorption changes seem to be caused predominantly by changes in the concentration of oxy- and deoxy-hemoglobin associated with metabolic and hemodynamic phenomena, although the activity of various cytochromes may also contribute. The time course of these phenomena is relatively slow, taking up to several seconds. The changes in scattering properties are less well understood. Studies on isolated neurons[9] and on the nervous systems of invertebrates[10] indicate that the scattering properties of neurons change during action potentials. This may be due to changes in the reflectivity of neuronal membranes; alternative explanations for these effects are also possible, and include volumetric changes or movements of ions across and around the membrane. Whatever the explanation for the scattering changes, it appears that they are more directly related (at least from a temporal point of view) to the activity of neurons than are the hemodynamic effects related to absorption changes.

NONINVASIVE NEAR-INFRARED OPTICAL IMAGING

The research reviewed in the previous paragraph described optical changes occurring in the brain during and after neural activity. For imaging purposes, it would be very useful to demonstrate that these phenomena could be detected noninvasively. The consideration that near-infrared light can penetrate deeply into living tissue has led various investigators to propose that this type of electromagnetic radiation might be used to image internal body structures.[11,12] The idea is that the optical properties of thick tissues with relatively high scattering and absorption coefficients (such as brain tissue) could be studied by measuring the parameters of the migration of near-infrared photons through the tissue. These parameters are differentially influenced by the scattering and absorption properties of the tissue itself.

Measurement of photon migration parameters of a particular area of the body can be done noninvasively by placing a light source (emitting light in the

near-infrared range—between 700 and 1300 nm) and a detector on two points on the surface of the skin, separated by a few cm. The parameters of interest are the proportion of photons reaching the detector (i.e., attenuation, or intensity) and the time taken by the photons to travel between the source and the detector (i.e., delay, or time-of-flight). Most tissues in the human head are highly scattering. For instance, recent estimates of the scattering coefficients of gray and white matters of the brain range between 16.1 and 28.7 mm^{-1} (for the gray matter) and between 133 and 228 mm^{-1} (for the white matter).[13] Although head tissues also absorb light, the absorption coefficient is estimated to be much smaller than the scattering coefficient: the typical value of μ_a in animal tissues is on the order of 1 mm^{-1}.[14] These basic optical properties of head tissues indicate that the Boltzmann's transport equation for photons inside the head can be solved in the diffusion approximation,[15–20] and that the propagation of photons through the tissue can be described as a diffusion process.[21] This has several consequences of practical importance.

First, the trajectory followed by the photons emitted by the light source and reaching the detector will be highly variable. Therefore, the envelope of the trajectories will be an extended volume: knowledge of this volume is very important, because only modifications in the optical properties occurring within this volume will be able to influence the measures, and conversely, the size (and, in particular, the width) of the volume will determine the spatial resolution of the technique. The smaller this volume, the better will be the spatial resolution of the measurements. If we consider *all* the photons emitted by the source that reach a detector, the volume within which they travel is fairly wide, yielding a very poor spatial resolution (on the order of several cm). However, spatial resolution can be markedly improved by selecting photons that travel relatively quickly between the source and the detector. This is because these photons travel, on average, along a relatively straighter path than photons that reach the detector more slowly. For this reason, procedures that select photons with a relatively short time-of-flight or delay ("time-resolved near-infrared optical imaging") will have a better spatial resolution than procedures that pool together photons with short and long delays ("steady state near-infrared optical imaging") (see Alfano, this volume).

Second, the diffusion process implies that photons propagate in spherical waves. This, in turn, implies that, if the measures were taken in an infinite medium, the average path followed by photons between two points in the media would be rectilinear. However, the presence of a surface boundary with a nonscattering medium may induce marked distortions in the average photon path. This is because, as we have already seen, the individual photons do not travel straight through the media: some of the photons travel close to the surface and are quite likely, in their random walk, to reach the surface, and to exit from the medium. In this case, they fail to reach the detector. Only photons traveling deeply into the medium are likely to reach the detector. As a consequence, the volume explored by a source-detector pair located on the

surface of the medium (head) is a curved spindle whose maximum depth is about one-half the distance between the source and the detector (in the case of dishomogenous media, this volume may be more complex). This theoretical prediction was confirmed experimentally using phantoms.[22] A practical consequence of this property of surface-bounded scattering media is that it is possible to study properties of relatively deep structures (such as the cortex) by placing a source and a detector on the surface of the head. Since the depth of the measured volume depends on the source-detector distance, the selection of the appropriate distance to be used in the measurements depends on the depth of the anatomical structure that needs to be imaged.

Third, the photon migration parameters are influenced by the source-detector distance: the number of photons reaching the detector (i.e., the attenuation) decreases exponentially with the source-detector distance, whereas the effect of source-detector distance on the delay parameter is linear. In the case of head tissues, the large attenuation of the light determined by the source-detector distance limits the maximum useful source-detector distance to less than 10 cm, and effectively limits the penetration of the technique to less than 5 cm from the surface. This has practical importance, because it makes full-head imaging of the adult human head impossible (unless the source or the detector were placed in some cavity inside the head such as the mouth).

NEAR-INFRARED EXPERIMENTAL APPARATUS

The earliest functional studies on the brain using photon migration methods were based on steady state optical systems, which involve measurements of the attenuation of light of particular wavelengths.[23–26] These systems are inexpensive and easy to use, but, as explained earlier, provide limited spatial resolution. A higher spatial resolution can be obtained with time-resolved methods, which allow the selection of fast-traveling photons. Photon delay can be estimated using "time-domain" methods[27] or "frequency-domain" methods.[28] Time-domain methods are very accurate, but have long acquisition time and are expensive. Frequency-domain methods are also quite accurate, but are less expensive and allow for fast data acquisition (up to 1 KHz). For this reason they are suited to the study of brain dynamics. The studies to be described later in this paper were based on a frequency-domain method derived from instruments developed for fluorescence decay kinetics.[29,30] It consists of a light source modulated at 112 MHz and of a heterodyned detector. The use of a heterodyning frequency (1 or 5 KHz, depending on the experiment) allows the translation of the signal into a frequency range that is appropriate for relatively inexpensive A/D converters. Phase delay is then measured using frequency-domain analysis methods. The final result is an accuracy of the phase measurement of about 0.1 degrees (out of 360 degrees). This corresponds to measurements of changes in photon delay with an accuracy of approximately 2.5 ps (10^{-12} seconds). This level of

accuracy is necessary for the measurement of 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 the experiments described, the light source was a light-emitting diode (LED) with a wavelength of 715 nm, and a power of less than 1 μW. The source-detector distance was fixed at 3 cm (computer simulations indicated that this distance allows for the detection of phenomena occurring at a depth of 0.5–2.5 cm).[31] Photon migration parameters were acquired using sampling rates varying between 12.5 and 50 Hz in different experiments. The measurements were obtained using a single-channel system (maps were obtained by repeating the observations at multiple locations). A multiple-channel system is presently under construction.

An important consideration in the study of optical parameters is the effect of vascular pulsation. The pulsation of the vascular system, and in particular of large and medium arteries, that is associated with systolic activity of the heart produces relatively large changes in the transmission of light through tissue.[32] This signal may, of course, be quite significant when the interest is in studying hemodynamic effects related to brain activity. However, when the interest is in visualizing directly the activity of neurons, this may produce substantial artifacts that are difficult to eliminate with simple filtering approaches. It is therefore important to estimate the contribution of this phenomenon to the observed data, in order to subtract it from the recordings or to study it separately. We have developed an algorithm for the estimation of this artifact based on a regression procedure in which the effect of systolic pulsation is estimated from the data.[33] The procedure takes into account beat-to-beat variability in pulse rate and amplitude. Our tests showed that, when used to estimate and subtract the effects of pulsation from the optical recordings, the procedure substantially reduces the impact of the pulsation artifact. This procedure is applied to all the data we have collected.

EXPERIMENTAL RESULTS OBTAINED USING NONINVASIVE NEAR-INFRARED TECHNIQUES

Path of Light and Effects of Skull

The purpose of these studies was to provide evidence that noninvasive optical imaging of deep structures enclosed in a highly scattering medium was possible,[12,22] at least for a depth of up to 4–5 cm. Our studies focused on two issues: (a) what is the volume explored using surface optical measures of photon migration? and (b) what is the influence of anatomical structures such as the skull on these measures? Some of the results of these initial studies were reported by Gratton *et al.*[22] These studies were conducted using simple phantoms simulating some of the optical properties of the human head. A semiinfinite scattering medium bounded by a nonscattering medium was simulated using a tank filled with skim milk. The path followed by photons migrating between a source and a detector located on the surface of the

scattering medium was explored by observing the effects of a small absorbing sphere immersed in the tank on the measurements. The placement of the absorbing sphere was varied systematically, and photon migration parameters were measured for each sphere placement. The reasoning used in this study was that the sphere interferes with the measurements only if it intersects the path followed by the photons. The results confirmed the prediction that photons traveling between the source and the detector follow a semicircular path. In addition, the data demonstrated that the volume explored by time-resolved measures was much narrower than that explored by steady state measures (see FIGURE 1). In a different experimental setup, we showed that images of the small sphere submerged in the milk tank could be obtained from surface measures. We further showed that the images could be obtained even through bony structures. These images were obtained by placing a small absorbing sphere inside a sheep's skull. The skull was then submerged in skim milk. The surface of the milk was scanned using a source-detector pair. The image obtained with the sphere inside the skull was compared with an image obtained without the sphere. The difference between the two images indicated that structures with specific optical properties can be visualized through the skull.

Noninvasive Measurement of Functional Optical Effects: Distinction between Fast and Slow Effects

During the last few years, several studies have shown that optical methods based on measures of photon migration parameters are sensitive to functional changes of brain activity.[23–26,31,34] This research has led to the identification of two types of changes: *fast effects* (with a 50– 500-ms latency) and *slow effects* (with a 2–10-s latency). These two effects can be distinguished in a number of ways. First, the two effects have very different time courses. Second, they are measured in different ways. Slow effects are best observed by using a sustained activation task, such as a task involving repeated stimulations (or repeated movements) so that the cumulated effects of a number of individual stimuli can be observed. This approach is similar to that used for fMRI and PET. Fast effects, on the other hand, are best visualized by averaging the time course of the activity elicited by (or associated with) individual experimental events (i.e., stimulations or movements). This approach is similar to that used for ERPs and MEG. For this reason fast effects can be studied in conditions in which different types of stimuli (or responses) are intermixed, while slow effects can be studied only in conditions in which stimuli of the same kind are blocked. Fast and slow effects also differ in terms of the patterns of optical effects observed. While slow effects can be observed using both steady state and time-resolved methods,[24,26,31] it appears, at least on the basis of our own observations, that fast effects are best visualized using time-resolved methods.[31,34] This may suggest that fast effects are more localized than slow effects. In agreement with this hypothesis, Monte Carlo simulations reported

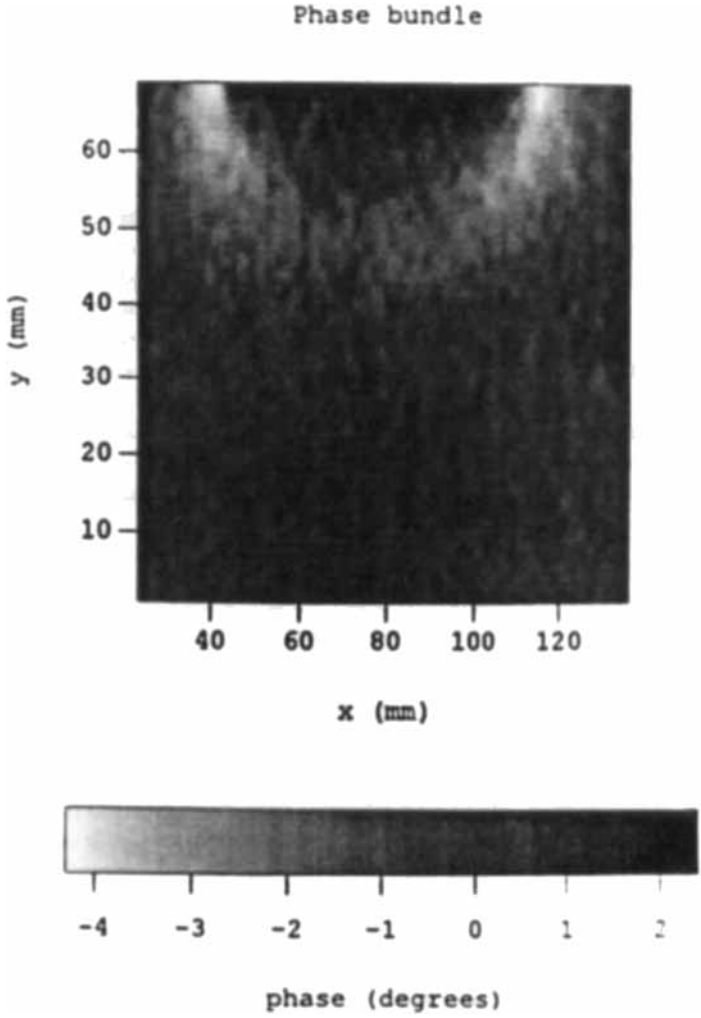

FIGURE 1. Empirical measurement of the volume influencing measurements of photon delay between two locations on the surface of an extended, surface-bound scattering medium, simulating the head. The measurements were conducted on a phantom (a tank full of skim milk). Near-infrared light was injected at the surface of the milk on the left end of the arc, and measured at the right end. The relevant volume was measured by systematically moving a small absorbing sphere in various locations within the medium. *White areas* indicate positions within the scattering medium in which the absorbing sphere will determine a reduction of the photon delay, and *black areas* indicate positions in which it will determine an increase in the photon delay. Note that the volume explored will vary if the position of the light source and/or of the detector is changed. Further details are given in Gratton *et al.*[22]

in Gratton *et al.*[31] suggested 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.

To a large extent, the slow effects can be attributed to oxygenation and hemodynamic changes that occur in active areas of the brain. Therefore, these effects appear to be related to the absorption effects studied with exposed cortex reflectivity measures and with the BOLD-fMRI signal.[26,35] They may also be related with the effects observed with PET studies of blood flow, although the time course of the latter cannot be studied with the same level of detail. In the remainder of this paper we will focus on experimental results related to the fast optical signal.

The Fast Optical Signal

We have carried out several studies using the fast optical signal.[31,34,36–38] These studies indicate that the properties of the fast signal are consistent with the hypothesis that it may reflect localized neuronal activation related to specific stimulation (or preparation for movement). Using the fast signal, we have obtained maps demonstrating spatial resolution of at least 5 mm. In further studies using visual stimulation we have obtained evidence that (a) optical activity can distinguish between stimulation effects (localized to area 17) and attention effects (that are evident in extrastriate areas of the occipital cortex); (b) the fast optical signal shows spatial correspondence with fMRI data and temporal correspondence with visual evoked potential data collected in the same paradigm; and (c) 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).

We will now describe in more details two of the studies we have conducted; other studies are described in other papers currently in preparation.

Tapping Task

Gratton *et al.*[31] recorded the fast optical signal (with detectors placed on the surface of the head directly above the two motor cortices, and the light source placed on the vertex) while subjects were performing a task requiring tapping with one hand or one foot at regular intervals. In this section we will review the data related to hand movements. Because of the neuroanatomical characteristics of the primary motor cortex (in which the right motor strip controls movements in the left part of the body and vice versa), differential effects were expected for hand movements that were contralateral or ipsilateral to the location of the detector. Specifically, it was hypothesized that when the detector was placed on the side contralateral to the hand movement, the

photon path should cross motor areas in which neurons were active. In this study, four subjects were asked to tap with one of their hands for 10 s at a frequency of 0.8 Hz. Ten seconds of rest preceded and followed the tapping period. As hypothesized, systematic differences in the photon delay parameter emerged as a function of movement side, consisting of changes occurring at the tapping frequency (0.8 Hz) and/or its harmonics.

Quadrant Stimulation Study

The tapping experiment described in the previous paragraph had several limitations. Specifically, maps of activity could not be derived from the data because only two locations (one above each of the motor strips) were tested. In addition, the data recording was not time locked with the tapping movements of the subjects. In the study described in this section we tried to address these problems,[34] in order to determine whether fast optical signals could be used to derive a retinotopic map of the primary visual cortex. To gain more understanding of the time course of the fast optical signal, a faster sampling rate (20 Hz) was employed, and the recording of the optical parameters was time locked to the presentation of visual stimuli. The stimuli consisted of four black-and-white vertical grids, displayed in the four quadrants of a computer monitor. On 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 in the segment of primary visual cortex being stimulated on each trial. Photon migration parameters (light intensity and delay) were measured from 12 scalp locations over the occipital lobe, to obtain a map of the visual cortex. Average waveforms corresponding to the changes in optical parameters during the first 500 ms after stimulation were derived. The analyses identified a deflection in the delay parameter, peaking 100 msec after stimulation and reaching a maximum delay of about 10 picoseconds (see FIGURE 2). The maps obtained with these data (reported by Gratton *et al.*[34]; see also FIGURE 3) indicate that the areas of maximum response for each of the four stimulation conditions correspond to the expected retinotopic map of the visual field in primary visual cortex (i.e., the maps are inverted along both the vertical and horizontal axes). Direct data on the depth of these effects are not available. However, simulations reported by Gratton *et al.*[31] 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 located at a depth of 1.5–3 cm (which corresponds to the depth of the superficial half of the primary visual cortex). The latency of the effects appear consistent with the idea that they may be due to the scattering changes ensuing in conjunction with electrical neuronal activity, as proposed by Stepnoski *et al.*[10] (see also Frostig[5]).

CONCLUSIONS

The results of the studies we have conducted using the fast optical signal (some of which were reviewed in this paper) suggest that this signal occurs simultaneously with or before the surface electrical potential (evoked potential or ERP). This makes the fast optical signal an ideal marker for indexing the occurrence of neuronal activity. The fast optical signal also appears to be

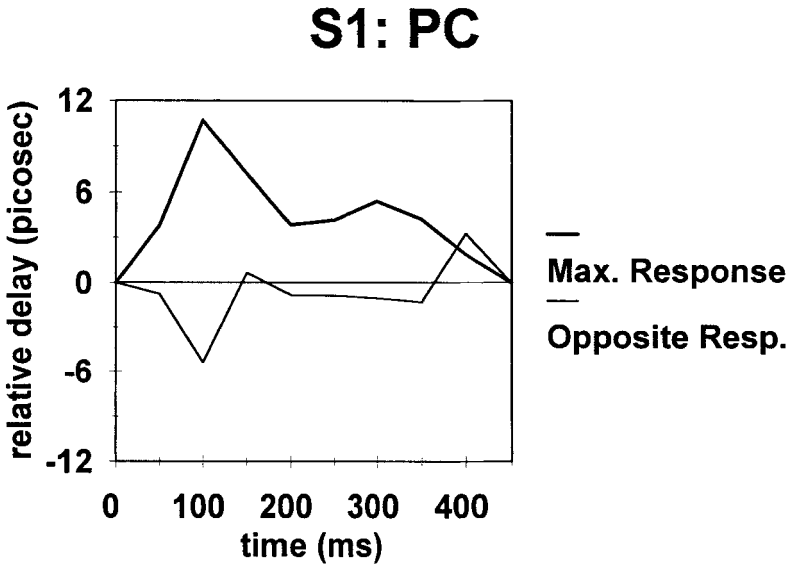

FIGURE 2. Time course of the fast optical signal elicited by grid reversals in occipital areas in a normal human subject. The measures were taken noninvasively, and indicate changes in photon delay with respect to a prestimulus baseline period. The data were averaged across four conditions in which different quadrants of the visual field were stimulated. Each condition was expected to stimulate a slightly different area of the occipital cortex. The *thick line* refers to the optical signal measured from the location at which response was maximum for each quadrant stimulation condition. The *thin line* refers to the optical signal measured from the same location when the opposite quadrant was stimulated. Note that the response peaks 100 ms after stimulation. Further details are given in Gratton *et al.*[34]

very localized, so as to allow one to distinguish between different parts of the same neuroanatomical area of the cortex (as in the case of Brodmann's area 17). Data we have recently obtained[38] indicate that this localization is consistent with that obtained using fMRI. This indicates that the fast optical signal is suited for examining the time course of neural activity in selected brain areas. This may be useful in two ways. On the one hand, it may help us study the relative timing of neuronal activity in different brain areas. This may have profound theoretical implications, since we expect most brain functions

(such as vision, memory, attention, language, movement control, etc.) to depend on the dynamic interactions between different brain areas. On the other hand, the possibility of deriving data with high resolution both in the temporal and the spatial domains may help integrate various noninvasive methods for studying brain function, and in particular electrophysiological and hemodynamic techniques.

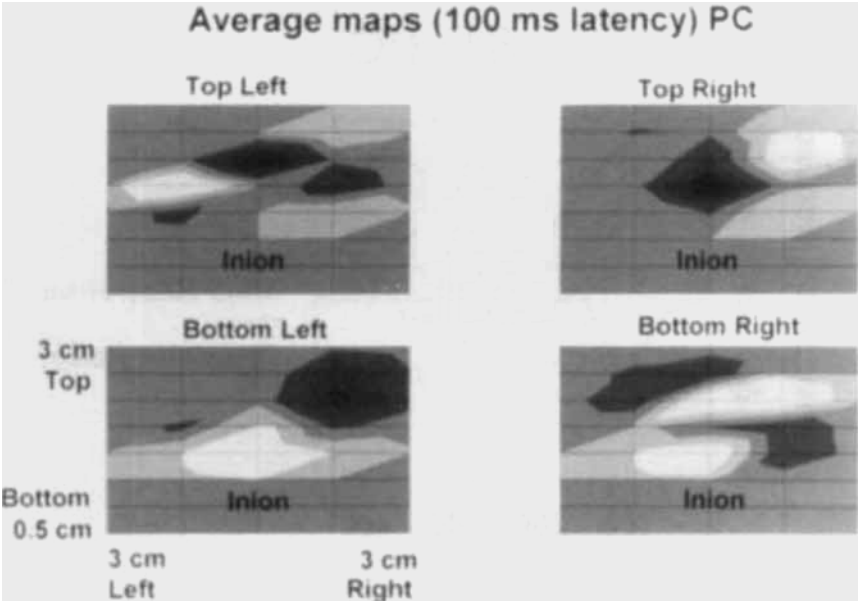

FIGURE 3. Maps of the fast optical signal elicited by each quadrant stimulation condition. The maps were obtained by scanning the surface of the occipital area of the head of a normal subject, over an area corresponding to the primary visual cortex. The *x*- and *y-axes* reflect horizontal and vertical distances from the inion, expressed in cm. *Black areas* indicate locations in which photon delay increased 100 ms after stimulation, and *white areas* indicate locations in which it decreased 100 ms after stimulation. The measurements are expected to be maximally influenced by the optical properties of brain areas with a depth varying between 0.5 and 2.5 cm from the surface of the head. The location of maximum increase in photon delay changes with the stimulation condition in a manner consistent with the contralateral, inverted representation of the visual field in primary visual cortex in humans. Further details are given in Gratton *et al.*[34]

Although noninvasive optical methods are quite promising, it is also clear that these methods are still evolving. Our data are still constrained by methodological issues. They include: (a) lack of a tridimensional reconstruction algorithm; (b) limits in the penetration into deep brain regions; and (c) limited understanding of the biophysical and physiological mechanisms responsible for the observed effects. Specific research addressing these issues

may open new possibilities for the application of noninvasive optical methods to the study of brain function.

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DISCUSSION

QUESTION: What are things that you might do to improve the resolution for the brain beyond even a half centimeter, and would the modulation frequency affect that?

E. GRATTON: You can increase the frequency. There is a direct relationship between frequency and penetration. The more you increase the frequency, the less you penetrate. So actually frequency can be better used to see more deeply or superficially. In answer to the question, at least in the simulation, a resolution of half a centimeter is really the ultimate resolution that you can have at the depth of the human brain.

QUESTION: So if you get closer to the brain, then you can improve the resolution?

E. GRATTON: Of course, if you can get closer; if you can be in contact, the resolution is just the optical resolution. But at the distance of around 3 or 2.5 centimeters resolution of half a centimeter is the best you can obtain using the diffusion photons. There is no way you can improve on that. I'm talking about the resolution. That doesn't pertain to localization. Localization, of course, is much, much better.

QUESTION: Actually there was a corollary question: when you said resolution, was that two-point resolution?

E. GRATTON: Yes, resolution in the classical sense that there are two points that you can separate and see as distinct points. This is what I mean by resolution. Localization, of course, is much, much greater. And, of course, that also assumes some sort of contrast, because the larger the contrast, the better the resolution.

QUESTION: To confirm some of your ideas, have you ever thought of going to the neurosurgery room when they opened the skull?

E. GRATTON: I have not been there.

QUESTION: Could you get some results with higher resolution and accuracy without the skull there?

E. GRATTON: It should be possible practically. But with the reflectivity measurements shown today, why would you want to get anything better? Those have essentially optical resolution.

QUESTION: So you think the skull isn't really important then? What are the brain changes that take place, and are they localized in the brain as compared to the skull? So you say that there are real changes at the level of the neurons and the movement of water that changes the scattering properties of the water?

E. GRATTON: I have all the time scales. Most of the things I say are unfortunately indirect and based more on consideration of the time scale of events. The color experiments are different from the one we did before. But if you look at the time scale here, clearly you can start to see the onset of the event after 20 milliseconds, for instance. Now, to think that those things happen at different levels in the water or in the cerebral fluid or blood flow—I think that is difficult to explain. And those are scattering changes, they're not absorption changes. This is, for the moment, the information we have. So there are scattering changes; and they occur, let us say, essentially simultaneous to the electrical signals as I showed, and they are fast.

QUESTION: And if one tries your optical system on a rat brain, for example, and stimulates at various frequencies, what's the source of the scattering changes? Are there water movements within membranes associated with the action potential itself? What I'm really asking is what is the basis for the scattering change that is associated with electrical activity?

E. GRATTON: Clearly, to me, this is the most important question, so I posed the question to myself many times, and I tried to discuss it with many people. So I am not underestimating your question. The explanation that we came out with is, first of all, changes in shape. You know, changes in volume will change the scattering. Changes in reflectivity due to ions, for example, on the membrane will also change the scattering. So there are a series of processes. We cannot tell you now which one is more important than another, but those are possible sources of changes in the scattering coefficient.

STANLEY RAPOPORT (NATIONAL INSTITUTE ON AGING, BETHESDA, MD.): I know from the work of Tasaki and Saropolis from the old days that the action potential itself is associated with volumetric changes in the membrane on the time scale because the current changes are associated with ion flow changes. Is that what you're saying is occurring?

E. GRATTON: Yes, that is the best hypothesis. That doesn't mean that we proved that at all.

QUESTION: I was just going to make the point that Dr. Rapoport just made—that Dr. Tasaki several years ago was studying volume changes in the isolated nerve as the action potential moved down in there, and that very well could contribute to scattering changes.

E. GRATTON: This is the most likely explanation.