Toward Noninvasive 3-D Imaging of the Time Course of Cortical Activity: Investigation of the Depth of the Event-Related Optical Signal

Gabriele Gratton,*1 Anita Sarno,* Ed Madin,* Paul M. Corballis,† and Monica Fabiani*

*University of Missouri at Columbia, Columbia, Missouri 65211; and †Dartmouth College, Hanover, New Hampshire 03755

Received March 30, 1999

INTRODUCTION

The past decade has seen a vast improvement in the technology available for studying human brain function in a noninvasive manner (Posner and Raichle, 1994; Toga and Mazziotta, 1996). Several noninvasive brain imaging techniques are now available, some excelling in their temporal resolution (such as electroencephalography, event-related brain potentials, and magnetoencephalography) and some particularly valuable because of the localization information they can provide (such as positron emission tomography and functional magnetic resonance imaging—fMRI).

The combination of spatial and temporal information has been advocated as an important tool for the analysis of the dynamics of brain function (Barinaga, 1997; Gratton and Fabiani, 1998). We have recently proposed that the event-related optical signal (EROS), a new method based on the measurement of changes in parameters of the migration of near-infrared (NIR) photons through the head, can provide images of brain activity which combine spatial and temporal specificity (Fabiani et al., 1996; Gratton et al., 1995a). In fact, the visually evoked EROS can measure phenomena that have a latency similar to that of visual-evoked potential (VEP) and a localization which is consistent with that of blood oxygenation level-dependent (BOLD)—fMRI effects elicited by similar stimuli (Gratton et al., 1997). However, the data reported in these studies were based on surface-projection maps and provided little specific information about the depth within the brain of the phenomena giving rise to the fast optical signal. In the present study we show that variations in the distance between the near-infrared light source and the light detector can be used to determine the depth of the optical effects related to brain activity.

Noninvasive optical imaging is based on the measurement of parameters of the migration of NIR photons through the head. These parameters include the number of photons (i.e., intensity) delivered by a source located on the surface of the head and reaching a detector located at a few centimeters’ distance, as well as the time taken by these photons to reach the detec-
tor (i.e., time of flight or phase delay). These parameters are influenced by the absorption and scattering properties of the medium (in this case, brain and other head tissues). Working on the exposed cortex of monkeys and other animals, Grinvald and his collaborators (Malonek and Grinvald, 1996; see also Frostig et al., 1990; Grinvald et al., 1986) have shown a number of changes in the optical properties of active brain tissue (intrinsic signal). They include both scattering and absorption effects. The absorption changes are, for the most part, related to changes in the concentration of oxy- and deoxyhemoglobin due to the exchange of oxygen between the blood and the tissue, as well as to the increased blood flow in active brain areas. These effects are relatively localized (within 1–2 cm from the active area) and their time course is delayed with respect to the neural activity by at least 0.5 s (and usually much longer). Several investigators have demonstrated that the absorption changes can be observed noninvasively in humans using NIR light of two or more wavelengths (Chance et al., 1997; Hirth et al., 1997; Hock et al., 1997; Hoshi and Tamura, 1993; Kato et al., 1993; Meek et al., 1995; Obrig et al., 1996; Wenzel et al., 1996).

The scattering changes follow more closely the activity of the neurons. Rector et al. (1997) reported that scattering changes may occur in hippocampal slices within 5 ms of evoked electrical activity. Using optic fibers implanted in the hippocampus of freely moving animals, they (Rector et al., 1995) also reported the measurement of optical activity with a frequency of 5–7 Hz, corresponding to the \( \delta \) band measured electrically. They attributed these changes to scattering phenomena. Other authors, including Malonek and Grinvald (1996) and Frostig et al. (1990), also reported fast scattering effects in brain tissue. The mechanisms responsible for these signals are not yet completely clear. Changes in the reflectivity of neuronal membranes during action potentials have been known to occur for a long time (see Cohen, 1972; Hill and Keynes, 1949).

In addition, Andrew and MacVicar (1994) have shown in vitro that osmolar changes, such as those due to the movement of ions and water through the neuronal membrane, can produce large changes in the scattering properties of neural tissue. They also showed that substitution of potassium with other ions which do not cross the neuronal membrane prevents the scattering changes associated with neuronal activity. Whatever the cause, it is clear that scattering changes may occur very rapidly in active brain areas, with a latency and time course similar to those of electrical phenomena.

Gratton et al. (1995a) reported data indicating that optical changes with a fast time course can be recorded noninvasively in humans. They presented to their subjects four vertical grids (one per visual-field quadrant) and stimulated one of the quadrants of the visual field by means of reversals of one of the grids. Using a 50-ms sampling rate, they found that the reversals elicited an optical response in occipital areas with a latency of about 100 ms. This response consisted of an increase in the time taken by NIR photons to travel through the head. Simulation studies carried out in a separate study indicated that this finding is consistent with a reduction in scattering and/or absorption in a layer located at least 1 cm deep within the head (Gratton et al., 1995b; for a discussion, see also Gratton and Fabiani, 1998). Gratton et al. (1995a) also showed that the localization of this response varied with the quadrant of the visual field that was stimulated, in a manner predictable on the basis of the inverted contralateral representation of the visual field in primary visual cortex. Taken together, these data are consistent with the idea that the EROS response is generated from within brain tissue, more specifically the cortex. In a subsequent paper, Gratton et al. (1997) showed that the time course of the EROS response was consistent with that of the VEP and that the localization of the response was consistent with that of the BOLD–fMRI signal recorded with similar stimuli in the same subjects. The EROS response to visual stimulation in occipital areas (i.e., an increase in photon delay with a latency of 60–100 ms from stimulation) has been replicated in several subsequent studies (Goodman et al., 1996; Gratton, 1997; Gratton et al., 1998a,b). An increase in photon delay with a time course parallel to that of electrical activity has also been observed in temporal areas for auditory stimuli (Rinne et al., 1999) and in motor areas in the period immediately preceding and following movements (Gratton et al., 1998).

Near-infrared photons diffuse randomly in scattering media, such as the brain and other head tissues. This limits the spatial specificity of the measures. However, for a given source–detector pair, there are regions of the head along a crescent-shaped volume that are more likely to be traversed by the NIR photons than others. Therefore, changes in these areas due to brain activity are more likely to affect the measurements. This allows for some degree of estimation, at least at a statistical level, of the location of the EROS effect (see Gratton et al., 1994; Gratton and Fabiani, 1998).

In principle, the statistical volume traversed by the photons can be reduced in diameter (and therefore the measure can be made specific to a smaller volume) by selecting photons which take a relatively short time to travel between the source and the detector (Alfano et al., 1997). This can be accomplished by using “time-resolved” optical measures. The term “time-resolved” indicates optical methods that allow for the measurement not only of the amount of NIR light emitted by the source and reaching the detector, but also of the time taken by the photons during this migration process. This time is proportional to the length of the path the photons have traveled. Therefore, by selecting photons with short time of flight, we are also selecting
photon paths that have traveled through short paths. These short paths tend to be relatively similar to each other, with the consequence that they describe, collectively, a relatively narrow volume. Whereas in theory this volume can be rendered very small (therefore yielding measures related to a very small, narrow volume), in practice it is very difficult to achieve spatial resolution on the order of 1 mm or less. In most actual cases, the cross section of the volume investigated will extend several millimeters (in some cases up to 1 cm or more). In addition, the statistical width of the crescent-shaped volume traversed by the photons depends on the optical properties of the medium itself (Arridge and Schweiger, 1995) and may therefore vary in different subjects and conditions.

A convenient procedure to obtain time-resolved optical measures is to modulate the light source at a high frequency (e.g., 110 MHz) and to consider the phase delay of the photon density wave reaching the detector (Gratton and Limkeman, 1984; see also Cooper et al., 1996; Cope and Delpy, 1988). Theoretical computations lead to the prediction that, at least for a homogeneous scattering medium (in which temporal variations in scattering and/or absorption can influence the phase delay measurement for a given source–detector pair) the statistical volume is shaped as a curved spindle, with vertices at the source and detectors and maximum depth approximately equal to half the source–detector distance and width depending on the optical properties of the tissue and on the frequency of modulation (Arridge and Schweiger, 1995; Sevick et al., 1994).

In a previous study, Gratton et al. (1994) tested this theoretical prediction in a phantom simulating a homogeneous, surface-bounded scattering medium. The results confirmed the expectations derived from theoretical computations. By using a number of source–detector pairs, data such as these could be used for precise localization of optical signals (see Toronov et al., 1998, for an application of this principle to the study of the movement of absorbing objects within scattering media), with a maximum theoretical accuracy of a few millimeters. However, the situation may be more complex in dishomogeneous media, like the head. In fact, the statistical path followed by the photons can be deformed significantly by the presence of highly scattering (such as the white matter) or absorbing structures (such as blood vessels). In addition, in the head there exist tissues with very low scattering coefficients, such as the cerebrospinal fluid contained in the subarachnoid space and in the ventricles. It has been hypothesized that these areas may provide a “path of low-resistance” for the NIR photons, which may therefore short-circuit the brain and the cortex. Some investigators have proposed that these dishomogeneities, and particularly the presence of cerebrospinal fluid in the subarachnoid space, might severely limit the penetration of NIR surface measurement (Okada et al., 1997). Subsequent simulation work by the same group (Firbank et al., 1998) has led to a modification of this hypothesis and to the prediction that noninvasive optical methods do reach superficial areas of the cortex, but only up to 4 mm from the surface of the cortex. In fact, the actual impact of the head dishomogeneities on the measurements is not well known, because the complexities of brain anatomy (both at a macroscopic and at a microscopic level) are difficult to model, and systematic over- and underestimations of the effects of dishomogeneities may occur (but see the work of Boas et al., 1994; Danen et al., 1998; Durduran et al., 1997). Further, postmortem data on the optical properties of various tissues (used in some studies) may be misleading because of the absence of blood circulation and of other phenomena related to tissue degeneration after death.

Therefore, in summary, there is evidence from functional studies that EROS effects originate in the cortex. Simulation studies suggest that, if large dishomogeneities were present, they may impair the penetration and depth localization power of the EROS measure. However, the actual impact of these dishomogeneities is not known, and the current study attempts to address some of these issues.

In the present paper we report an empirical test of the propagation of NIR light into the head tissue. Our approach is based on using an experimental manipulation that is expected to produce variations in the depth of the area of the visual cortex that is activated, namely a manipulation of the eccentricity of visual stimuli along the horizontal axis (e.g., Maclin et al., 1983). We recorded both EROS and fMRI from the same subjects using the same paradigm to provide an external estimate of the depth of the cortical area involved. We measured the EROS effect (i.e., increase in phase elicited by the stimulation with a latency of 60–80 ms) using several source–detector distances. Following our previous studies carried out on homogeneous phantoms (Gratton et al., 1994), we predicted that the source–detector distance for which the effect should be maximum would depend on the depth of the cortical area involved. More precisely, we predicted that the depth of the activated area would be equal to half the source–detector distance for which the optical effect was maximum.

**METHODS**

**Subjects**

Nine healthy volunteers (four females, ages 21–46, mean 27.9) were run in the experiment after signing informed consent and were paid for their participation. All subjects were right-handed and reported normal or corrected to normal vision. EROS data were obtained.
from all subjects, whereas fMRI data were collected from seven subjects (four females, ages 21–46, mean 28.3). The two subjects excluded from the fMRI portion of the study could not be placed in the scanner due to counterindications.

Stimuli and Procedures

The stimuli consisted of brief flashes of two grids presented to the left and right of a central fixation cross. In different blocks, the grids varied in eccentricity and size. Size was varied to compensate for the phenomenon of cortical magnification, so that approximately equal areas of the cortex would be activated in different conditions. Four eccentricities were used: 1, 2, 4, and 8° of visual angle with respect to the central fixation cross. The grids were square, with each side of 1, 2, 4, and 8° (i.e., the size of the grid equaled the eccentricity). All grids were formed by four vertical black bars and four vertical white bars, all of equal size (see Fig. 1).

The grids were flashed for 34 ms every 200 ms (5-Hz stimulation rate), for a total of 16 s. A given eccentricity/size grid was flashed within each 16-s block. Blocks were separated by 24 s of rest. The stimuli were presented on a CRT display during the EROS recording sessions and retroprojected on a translucent screen using an LCD projector (power: 800 lumen) during the fMRI recording session. During the EROS sessions, a chin rest was used to ensure an appropriate eye-screen distance. During the fMRI session, the subject was provided with a mirror to afford view of the translucent screen, and distances between the eyes and the screen and projector were kept fixed. There were a total of 32 blocks during the optical session and 12 blocks during the fMRI session.

There were two EROS recording sessions, run back to back on the same day. In each session, different locations of the occipital area were explored. For each location, data were obtained during 16 blocks (4 for each eccentricity condition). The different eccentricity conditions were interspersed following a repetitive pattern repeated four times (2, 8, 1, 4°). A similar pattern (but with each stimulation condition repeated three times before moving to a different stimulation condition) was used in the fMRI session. Subjects were instructed to fixate the central fixation cross during each block.

EROS Recording and Analysis

The optical recording was carried out using an OMNIA multichannel optical recording system (ISS, Inc., Champaign, IL). Data were recorded from 8 channels for each of the two sessions, for a total of 16 channels. The 8 channels were derived from different combinations of four sources and two detectors (of the six source placements and four detector placements available—see Fig. 2). The four sources used in each session were placed in a square (whose side was approximately 1 cm) with the left side placed approximately 4 cm to the left of the midline (see Fig. 2). The two sources in the middle row were used in both sessions. In the first session the four top sources were used. Of these, the highest were located ~3 cm above the inion, and the inferior sources were placed ~2 cm above the inion. In the second session, the four bottom sources were used. Of these, the top sources were placed ~2 cm above the inion.

FIG. 2. Schematic representation of the EROS montage. (A) The possible placements of sources (leftmost columns) and detectors (rightmost columns) with respect to the occipital cortex. (B) Examples of a short (S), medium (M), and long (L) source–detector distance. (C) The schematic relationship between source–detector distance and estimated depth of the photon path (and, therefore, of the depth of the predicted effect).
inion and the inferior sources were placed ~1 cm above the inion. The detectors were placed on a line at middle height between the inferior and the superior sources, in each of the sessions. In other words, the top two detectors were used in the first session and the bottom two in the second. One of the detectors was placed on the midline, and the other detector was placed ~1 cm to the right of the midline. This placement was used to scan an occipital area that, on average, corresponds to the surface projection of the primary visual cortex (area 17) of the left hemisphere (Maier et al., 1987). This was also one of the areas in which previous studies indicated the occurrence of an EROS response, characterized by an increase of phase delay with a peak latency of approximately 60–80 ms after stimulus onset (Goodman et al., 1996; Gratton, 1997; Gratton et al., 1995a, 1998a). The 16 channels encompassed three different source-detector distances, which were approximately 3 cm (or short, between the leftmost sources and the rightmost detector), 4 cm (or medium, between the leftmost sources and the leftmost detectors as well as between the rightmost sources and the rightmost detectors), and 5 cm (or long, between the leftmost sources and rightmost detectors) (see Figs. 2B and 2C).

The sources were 400-μm optic fibers connected to laser diodes emitting light at 750 nm with a power of 1.5 mW. The laser diodes were multiplexed, and their power was modulated at 110 MHz. Each laser diode was switched on for 5 ms every 20 ms (sampling rate 50 Hz). The detectors were 3-mm fiber optic bundles connected to photo multiplier tubes (PMTs). A 110.005-MHz current was inserted into the PMTs to permit measurement of the oscillations of the photon density wave at a cross-correlation frequency of 5 kHz (Gratton and Limkeman, 1983). The outputs of the PMTs were then sampled simultaneously at 40 kHz using an A/D card. The relative phase delay of the 5-kHz cross-correlational optical signal was then determined using fast Fourier transform. As a result of this process, a phase-delay estimate was obtained every 20 ms for each source-detector channel. The phase-delay data were obtained continuously during each block, starting 20 ms before the first flash.

Analysis of the optical data was conducted by first compensating for the pulsation artifact according to a procedure described by Gratton and Corballis (1995). Data were monitored for large, obvious artifacts (defined as data more than 3 standard deviations from the average observations for a particular subject and recording condition) due presumably to large movements of the subject, temporary drops in the light, and so on. These artifacts occurred in a very small proportion of the trials (less than 2%), which were discarded automatically and blindly prior to further analysis. The data for each block were then segmented into 200-ms epochs, starting 20 ms before each stimulus, and sig-
FIG. 3. Functional MRI data from one slice of one subject for the 1, 2, and 4° conditions. Areas with the most significant activity (increase in the BOLD response with respect to the interstimulation period) are shown in yellow ($Z > 4$) and red ($Z > 3$).

FIG. 4. Differences between the predicted and control EROS waveforms for all eccentricity conditions for three of the subjects. The time scale (abscissa) is expressed in ms ($0$ = stimulus onset). The values presented (ordinate) are phase-delay changes with respect to a baseline value recorded around the time of stimulus onset. The raw (unstandardized) data are presented on the left column (the scales for different subjects in this column are adjusted so as to be on a similar scale for different subjects and conditions). The standardized data (data obtained by dividing the average value obtained for each subject, eccentricity condition, individual recording channel—four channels were used for each distance—and data point by the standard error of the same value computed across subjects) are presented in the middle column. The standardized data averaged across different eccentricity conditions are presented in the right column.
RESULTS

fMRI

An example of the fMRI data obtained from one subject is reported in Fig. 3. In most subjects and conditions (22 of 28, 79%) there was an area in the medial occipital cortex for which the BOLD signal exceeded the established criteria \((Z > 3)\). The depth of the fMRI effects from the surface of the head are presented in Table 1. The data indicate, as expected, that the depth (i.e., distance from the surface of the head) of the activated area increased with eccentricity, reflecting the well-known organization of the visual cortex. A regression analysis was performed between the observed data and a model assuming that the depth of the activated area was linearly dependent on the logarithm of the eccentricity (Schwartz, 1980). The Pearson’s product-moment correlation between the depth predicted by the model and that observed was equal to \(0.52, t(20) = 2.71, P < 0.05\) two-tailed.²

These fMRI data indicated that the experiment succeeded in generating activity with a depth which varied systematically as a function of stimulus eccentricity. The results are consistent with the interpretation that the BOLD response was due to activity in the primary visual cortex (area 17), although it is also possible that other areas might contribute to the response as well.

EROS

The EROS response was analyzed in two different ways. The purpose of the first analysis was to verify whether the depth of the EROS response varied systematically with stimulus eccentricity, as predicted on the basis of our experimental hypothesis. It also served to identify the latency of the effects. For this reason, we compared the average EROS waveforms obtained at the three source–detector distances used under different eccentricity conditions. We expected that for the 1° stimulation condition, the response would be larger at the shortest source–detector distance, while for the 8° stimulation condition the response would be larger at the long source–detector distance, with intermediate values for the other conditions. We therefore separated the data into two groups: those for which we expected a response (experimental, or predicted, conditions) and those for which we did not expect a response (control conditions). The experimental conditions included the short source–detector distance for the 1° stimulation condition, the short and middle source–detector distance for the 2° stimulation condition, the middle and long source–detector distance for the 4° stimulation condition, and the long source–detector distance for the 8° stimulation conditions. The control conditions included all the other waveforms not included in the experimental conditions. In other words, the control conditions for the 1° stimulation would be the wave-...

² This analysis has some limitations, because the degrees of freedom are overestimated. This is partly compensated by the underestimation of the within-subject variance. A difference approach is presented in the section on the analysis of EROS.
forms obtained at medium and long source–detector distances and so on. This allowed us to compute an experimental and a control waveform for each subject. Examples of the difference waveforms from all distances for some of the subjects in the four stimulation conditions (1, 2, 4, and 8° of eccentricity) are presented in Fig. 4. The differences between experimental (predicted) and control waveforms averaged across the nine subjects and all stimulation conditions are presented in Fig. 5.

These data indicate that there was an increase in phase delay (EROS) in the predicted conditions with a peak latency of 80 ms. A smaller decrease in phase delay was observed at these latencies in the control conditions. The increase in phase delay observed in the predicted condition was consistent in polarity and latency with our previous observations (Goodman et al., 1996; Gratton, 1997; Gratton et al., 1995a, 1998a). The difference between the predicted and the control condition (shown in Fig. 5) was significant, t(8) = 2.20, P < 0.05 (one-tailed). The amplitude of the EROS response for each eccentricity condition and source–detector distance is shown in Fig. 6.

The purpose of the second analysis was to derive estimates of the depth of the EROS effects in order to compare them with the estimates of the depth of the BOLD–fMRI effects. Therefore, it was run in a manner similar to the analysis of the BOLD–fMRI response. It was based on the observation (from this and previous studies) that the EROS response occurs at a latency of approximately 60–80 ms after stimulation. Therefore we quantified the EROS response as the average standardized phase value for an interval between 60 and 80 ms with respect to the prestimulus baseline value. These responses were computed for each subject, eccentricity condition, and source–detector distance. Then, for each eccentricity condition, we identified the source–detector distance showing the largest response exceeding a minimum criterion, set at a standard score of 0.7.3 As was the case for the BOLD–fMRI signal, in some conditions there was no response exceeding the preset criterion. The results are shown in Table 2.

Note that, in this table, the depth estimates are based on a value equal to half the source–detector distance in millimeters for the source–detector pair showing the maximum response. This was considered to be a preliminary estimate of the depth of the EROS effect, based on the simplistic assumption that the head volume most sensitive to changes in optical properties would be shaped approximately as a semicircle. This prediction was based on a previous phantom study, carried out on a homogeneous medium, in which we observed that the crescent volume explored by phase-delay data reached an average depth of 0.48 of the source–detector distance (Gratton et al., 1994). Note that the EROS effect of course could occur at a depth that was more superficial than the maximum depth reached by the path of light. However, if this were the case, then the effect should be maximally visible at a shorter source–detector distance.

Table 2 indicates that an estimate of the depth of the optical effects could be obtained in 19 of 36 cases, suggesting that the EROS estimates may be more difficult to obtain than the fMRI estimates. The table also indicates that the average estimate of the depth of the optical effects steadily increased with stimulus eccentricity. To evaluate the significance of this observation, we used a procedure similar to that used for the fMRI and determined the fit of a model assuming that the depth of the optical effect was proportional to the logarithm of the eccentricity of the stimulus. The correlation between this model and the individual depth estimates was r = 0.57, t(17) = 2.81, P < 0.05 (two-tailed).

The regression analyses presented above for fMRI and EROS are problematic because some of the observations were repeated on the same subjects. This may lead to an overestimation of the degrees of freedom, which may be partially compensated by an underestimation of the within-subject variance (see Footnote 2). Therefore we ran two further analyses. The first was based on computing the correlation between the logarithmic model and the observed depth of the effects.
averaged across subjects. The correlation coefficients were very high, \( r = 0.99 \) for the fMRI depth estimates and \( r = 0.97 \) for the EROS depth estimates, in both cases corresponding to \( P < 0.05 \), with 2 degrees of freedom. This analysis, however, does not reflect the variability of the effects across subjects. Therefore we ran a second analysis to determine whether there was a linear trend in the effects. Since there were several missing cells, a traditional trend analysis could not be performed. In its place, we ran the following analysis. For each individual subject we computed a “trend” index \( y \) on the basis of the formula

\[
(1) y = \sum (w_i x_i) / \sum w_i^2
\]

where \( x_i \) are the differences between the depth estimates for each eccentricity condition \( i \) for that subject and the overall average depth estimates, computed across subjects and conditions, and \( w_i \) are weights associated with a linear trend \((-3, -1, 1, \text{ and } 3, \text{ respectively, for the four eccentricity conditions})\). The sums were computed by considering only the cells for which depth estimates were available. We then determined (using a t test) whether the average trend index, computed across subjects, was greater than 0. Quadratic and cubic trends were also analyzed using the same procedure but different sets of weights (respectively \(-1, 1, 1, -1, \text{ and } -1, 1, -1, 1\)). Note that this procedure is equivalent to a traditional trend analysis, with the following exceptions: (a) the overall mean across subjects is used instead of the mean for each individual subject to determine the deviation scores (this results in a loss of statistical power but is required because of the large number of missing cells) and (b) the missing cells are ignored in the analysis. The linear trend was significant for both the fMRI depth estimates, \( t(6) = 2.88, P < 0.05 \), and the EROS depth estimates, \( t(8) = 2.93, P < 0.01 \) (both one-tailed, since a positive trend is expected). The quadratic and cubic trends did not reach significance for either the fMRI or the EROS depth estimates (all t's < 1.2). These results are consistent with the logarithmic model presented above and indicate that both fMRI and EROS are able to discriminate between the occipital brain areas associated with different stimulus eccentricities.

**Relationship between EROS and fMRI**

The relationship between the depth estimates of the activated brain areas for each eccentricity condition obtained with fMRI and EROS are shown in Fig. 7 in the form of a scatter plot, in which the x axis refers to the fMRI estimates and the y axis refers to the EROS estimates. The diagonal line indicates the points for which the two estimates would coincide and provides a frame of reference for the values actually observed. Also presented in Fig. 7 are standard error bars for each type of estimate. Note that the two average functions are highly correlated \( (r > 0.99) \) and that the mean squared difference between the two sets of average estimates is smaller than 1 mm \((0.56 \text{ mm})\).

The data presented in Fig. 7 illustrate the consistency between the estimates of the depth of the activated area obtained with EROS and fMRI. In all cases, the discrepancy is well within the error bar. Further, the error bars for the EROS estimates are similar in size to those for fMRI estimates. In fact, the average standard error (across eccentricity conditions) for the depth estimates obtained with EROS is 1.5 mm, whereas that for fMRI is 1.6 mm. Taken together, these data suggest that the homogeneous head model used for evaluating the depth of the active brain area obtained with EROS provides results that are as reliable as those obtained with fMRI, although they can be obtained only in a subset of cases. Furthermore, the fMRI data provide an external validity check for these estimates. The data from Tables 1 and 2 and Fig. 7 indicate that this validity is very high \( (> 0.98)\).

Finally, it is important to note that the optical and fMRI estimates of the depth of the activated areas for each condition shown in Fig. 7 are averaged across subjects. We also computed the root mean square difference based on the individual subjects' estimates. The value of this difference was 6.9 mm, which was less than the sum of the spatial sampling rate of the two techniques.

**DISCUSSION**

The results of this study provide support to the hypothesis that variations of the source–detector distance can be used to evaluate the depth of the brain
area responsible for the EROS effect, albeit in a subset of the available conditions. Further, they suggest that a simple procedure, consisting of selecting the source-detector distance with the largest effect (and then halving this distance), may be used, at least in some cases, to provide reasonably accurate estimates of the depth of the EROS effects. The EROS data appear to get less reliable with increasing depth, which can be attributed to the reduction in photon penetration with depth (and therefore decreased signal-to-noise ratio).

The design of the study had three main features: (a) the use of three different source distances; (b) the use of four different stimulation conditions, assumed to produce activity in cortical areas differing in depth; and (c) the use of an external validation check of our procedure by measuring the BOLD–fMRI response in the same subjects and conditions.

Analysis of the fMRI response indicated that our manipulation was effective and that the depth of the effect varied by approximately 1 cm between the most medial and the most peripheral stimulation conditions. This result was of course predicted, since previous studies (e.g., Engel et al., 1994) have shown that the localization of the BOLD–fMRI response varies with the eccentricity of the stimuli and have provided support for the claim that fMRI can be used to derive detailed functional cortical maps. Although it is difficult to estimate the exact accuracy of the fMRI estimates in our study, the comparison with the logarithmic model and the between-subject variability suggest that, across subjects, it is possible to reach a localization power of less than 2 mm. This is in accord with previous findings (e.g., Engel et al., 1994). In our work, estimates of the depth of the BOLD–fMRI effect could be obtained in most cases from the 2, 4, and 8° conditions. However, the estimates for the 1° condition could be obtained in only two of seven subjects. It is possible that this may reflect an increased noise level for cortical regions that are very close to the subarachnoid space. Clearly, even more accurate localization estimates of the BOLD effect have been reported in the literature by using more powerful scanners and more advanced technology than those used in the current study (e.g., Menon et al., 1997). However, there may be a physiological limit to the accuracy of the measures which may be due to the precision of the neurovascular coupling (see Malonek and Grinvald, 1986). Finally, below subjects in anatomy and functional localization may play an important role in this respect.

As for the BOLD–fMRI signal, the EROS response also showed systematic differences between channels as a function of the eccentricity of the stimuli. These differences peaked at approximately 60–80 ms after each flash, suggesting that the EROS is related to a very rapid change in optical properties in the active cortical areas. The latency of this peak is quite similar to the EROS peak latency we reported in other visual stimulation studies using different conditions and interstimulus intervals (Goodman et al., 1996; Gratton, 1997; Gratton et al., 1995a, 1998a). The short latency of the EROS response suggests that this signal reflects some phenomenon which is closely related to the activity of the neurons. Indeed, various investigators have identified scattering changes which occur very rapidly after neuronal stimulation (e.g., Rector et al., 1997).

The main purpose of this study was to determine whether different source-detector distances could be used to estimate the depth of the area of the brain responsible for the EROS effects. Phantom studies based on homogeneous models suggest that this may be possible (e.g., Gratton et al., 1994), and similar conclusions can be drawn from Monte Carlo simulations also based on homogeneous models (e.g., Gratton et al., 1995b; Okada et al., 1997; Sevick et al., 1994). However, some investigators (Okada et al., 1997; Firbank et al., 1998) have pointed out that optical properties (absorption and scattering coefficients) of the various head tissues vary widely and that therefore a homogeneous model is inadequate. These investigators have used Monte Carlo simulations, phantoms, and theoretical models to argue that the penetration of NIR light into the head is severely limited if a layer with very low scattering and absorption coefficient is interposed between the superficial skull layer and the deeper brain areas. Okada et al. considered that the cerebrospinal fluid contained in the subarachnoid space may represent such a layer. This fluid would provide a low-resistance path for the photons. As a consequence, very few photons would penetrate into the scattering layers of the brain (cortex and white matter). The conclusions of this original work were later modified by successive simulations from the same group (Firbank et al., 1998) indicating that some degree of penetration of the cortex may be possible (up to 4 mm). However, these results, in particular those of Okada et al. (1997), appear to be partially in conflict with those presented here.

A corollary of the idea of short-circuiting of NIR photons through the subarachnoid space, presented in Okada et al. (1997), is that the penetration of the photons would be substantially independent of the source-detector distance (at least for distances greater than 2.5 cm). In fact, since this fluid is nonscattering, the photons could be expected to propagate along straight lines within the subarachnoid space. Therefore, increases in source-detector distances would produce minimum increases in the phase-delay parameters for source-detector distances exceeding 2.5 cm. Further, since this medium is clear, there would be a relatively small reduction in the photon transmission with distance.

Although quite sophisticated, these phantom models and Monte Carlo simulations are themselves based on
simplifications of the macro- and microanatomy of the various brain structures involved. For example, the cerebrospinal fluid is contained within the subarachnoid space that, unlike a clear layer, also contains an extensive network of blood vessels that are likely to be highly absorbing (Franceschini et al., 1998). Accurate modeling of a real, functioning head may be difficult. Therefore we believe that a true test of the actual penetration of noninvasive optical techniques for studying brain function needs to be carried out in vivo, that is, on the head of a normal, behaving person. In vivo studies carried out on infants by other investigators (Franceschini et al., 1998) have reported data suggesting that variations of the source–detector distance above 2.5 cm may actually influence the phase-delay parameter in a pronounced manner. These results conflict with the predictions of the original model by Okada and colleagues (1997) that uses a clear layer to simulate the subarachnoid space. In addition, Franceschini et al. (1998) found that light reaching the detector attenuates with distance to a much greater extent than what would be predicted on the basis of a model with a clear layer. However, these data were obtained on infants and therefore in conditions that may be considered different from those present in the adult brain.

The instrumentation used in the present study prevented us from obtaining absolute measures of the phase-delay parameter. However, it is possible to measure the attenuation of the light reaching the detector as a function of the source–detector distance. This value can then be compared with the result reported by Franceschini et al. (1998, Fig. 5a, p. 235) for infant heads. From that figure, it can be inferred that light propagating through the head of infants attenuates with source–detector distance by a factor of approximately 6.8/cm, an attenuation factor which far exceeds that occurring in a model containing a clear layer (in which light attenuates by a factor of approximately 3.7/cm). In our experiment, the data (averaged across subjects and experimental conditions, N = 9) indicate that light attenuates by a factor of approximately 7.9/cm (with a 95% confidence interval of 5.6–11.3/cm). Thus, our data indicate that light propagates through adult heads in a manner which is much closer to the situation occurring in the infant head than to that predicted by models containing a clear layer. It should be noted, however, that these data (as well as those of Franceschini et al., 1998) are not necessarily inconsistent with a limited penetration of NIR light in the cortex, as predicted by the subsequent modeling work of Firbank et al. (1998), which has suggested that some of the photons may penetrate the cortical tissue (up to a depth of 4 mm). In this case, since the cortex is a scattering tissue, a significant dependence of the optical parameters on the source–detector distance should be expected.

Although theoretically significant, this finding (as well as those reported by Franceschini et al., 1998) does not provide direct information about the depth explored by the optical measures or on whether different source–detector distances can be used to estimate the depth of EROS effects within the brain. Our approach to this problem was to generate real life conditions in which the activated area of the brain varied systematically. We could then directly test the hypothesis that the source–detector distance influences the depth within the brain that is explored by EROS measures by comparing signals obtained using different source–detector distances for each experimental condition. Since we also recorded fMRI in the same conditions, we did not need to make any a priori assumptions about where the phenomenon is occurring within the brain. We also did not need to make a priori assumptions about the way photons propagate through the head. However, the data could be used to test the validity and accuracy of depth estimates obtained with a simple algorithm derived from previous studies based on homogeneous models (Gratton et al., 1994; see also Arridge and Schweiger, 1995; Sevick et al., 1994).

Our results indicate that the source–detector distance that was most sensitive to the stimulation varied systematically in a fairly predictable manner. They indicated that short source–detector distances were most sensitive to more medial visual stimulation conditions, and longer source–detector distances were most sensitive to more eccentric stimulation conditions. Further, in some proportion of the cases, the data indicated that the source–detector distance showing the largest EROS effect could be used to estimate the depth of the activated area. A limitation of this procedure was that definite estimates could be obtained in only a little more than 50% of the cases. It also appears that estimates are more easily obtained when the effect is superficial (67% of the cases for the 1° condition) than when the effect is deeper (33% of the cases in the 8° condition). This probably reflects the large noise level associated with the long source–detector distances, which in turn is a reflection of the small number of photons reaching the detector in this condition (on average, about 60 times fewer than for the short source–detector distance).

The data indicate that our algorithm, based on previous studies on homogeneous models, provides a reasonably accurate estimate of depth, at least for visual cortex. The differences between the depth estimates obtained with this very simple algorithm, requiring a minimum of computation, and those obtained with fMRI are small. The EROS–fMRI differences for the average estimates were less than 1 mm for each of the four eccentricity conditions. Larger differences were observed for individual subjects and conditions. However, they typically remained within the sampling error of the measurements. These data provide support
for the use of multiple source–detector distances to determine the depth of EROS effects, but they will need to be validated for other areas of the brain.

Our data suggest a relatively good localization power for EROS. This may apparently contrast with the notion that the movement of NIR photons through the head can be described as a random diffusion process and that the area explored by the measures is a relatively extended volume. However, to understand this apparent contrast, a distinction should be made here between spatial localization and spatial resolution. Localization refers to the use of a particular method to determine the place in the brain where the center of a particular focus of activity occurs, whereas resolution refers to the use of a particular method to measure separately activity from adjacent structures or to determine the extent of the active area. Some procedures may possess good spatial localization power while lacking spatial resolution. This can be particularly evident when there are extended sources of activity located in close proximity to each other. Spatial localization may, in some cases, be improved by averaging across trials, subjects, and conditions, in particular in the case of isolated, small sources, whereas this is not necessarily the case for spatial resolution. The present experiment does not address the issue of the spatial resolution of EROS, but only that of its localization power, in particular with respect to the depth of the activity. Therefore, the actual spatial resolution of EROS needs to be addressed in future studies.

Some of the data of the present study rule out an alternative interpretation of the EROS effect, which attributes this phenomenon to movements of the gray matter within the head or to changes in absorption due to hemodynamic phenomena. The first interpretation is in conflict with the fMRI results, which do not show any evidence of such movements. In particular, if brain movements pushing the gray matter closer to the surface of the head did occur, the fMRI data would show a movement artifact in the posterior region of the head which would be present for all conditions in the same area (the border between gray matter and the subarachnoid space). In addition, the time course of the activity does not suggest a hemodynamic or brain movement origin, since the effects occur regularly approximately 80 ms after each stimulation (with a repetition rate of 200 ms) with a return to the original state before the next stimulation (see Fig. 5). Further, it is unclear why the presumed brain movements should depend systematically on stimulus eccentricity and produce differential effects as a function of source–detector distance. Finally, the alternative interpretations cannot account for the close relationship between optical and fMRI data.

A limitation of the present study is that we investigated only effects whose maximum depth from the surface of the head (as estimated using fMRI) was less than 30 mm. These data, therefore, do not allow us to determine whether EROS can accurately measure and localize brain activity originated at greater depths. A problem with using EROS to study brain events that occur at a depth greater than 3 cm from the surface of the head is that the number of photons traveling between a source and a detector decreases exponentially with the source–detector distance. Therefore, the data are likely to become more and more noisy with increased distances. Given the relationship between distance and depth demonstrated in the present study, effects at depth greater than 3–5 cm may be very difficult to measure with the present instrumentation. In addition, the present study demonstrates that the homogeneity approximation works reasonably up to a depth of approximately 30 mm. However, it is not clear that it will work similarly well for effects located at greater depths. Further studies are required to explore these issues.

The simple procedure for depth estimation presented here is not based on a tridimensional reconstruction of the optical effects through the head. In fact, the procedure used is merely a localization method, whose validity depends on the assumption that activity within the focal area investigated by the optical measures can be ascribed to a small volume. In many cases this assumption is probably not appropriate. In these cases, a tridimensional reconstruction algorithm, possibly based on an appropriate model of the propagation of photons through the media, is required. Several investigators have proposed mathematical algorithms for tridimensional reconstruction of optical parameters (Arridge and Hebden, 1997; Arridge and Schweiger, 1997; Barbour et al., 1994; Chang et al., 1997; Paulsen and Jiang, 1995; Zhu et al., 1997). In order to be useful, all these methods require a large number of sources and detectors—many more than those available in the present study. However, the present data can be useful in designing an appropriate tridimensional reconstruction procedure. First, the data indicate that multiple source–detector distances can be very useful to derive depth information, an indispensable aspect of tridimensional reconstruction. Second, the data suggest that departures from homogeneity may play a relatively minor role in the propagation of photons through the head tissues. If this were to be confirmed for other cortical areas, homogeneous models could be used for tridimensional reconstruction with a limited error. These models are computationally much simpler than dishomogeneous models and require less a priori information about the specific anatomy and optical properties of head tissues. Thus, the problem of tridimensional reconstruction of optical data may be simplified without greatly sacrificing accuracy. This hypothesis needs direct verification. The approach used in the present study may be particularly useful for this purpose, in that it shows how the active region of the brain...
can be accurately controlled using appropriate stimulation and fMRI activation maps. Note that situations with multiple regions of brain activity could be generated easily by presenting simultaneously stimuli in different regions of the visual field. In this fashion the effect of the interaction between different active areas could also be studied in detail.

In summary, our data support the claim that source-detector distance is a primary determinant of the depth reached by the photons within the visual cortex and possibly in other regions of the head. Further, the data suggest that, at least as a first approximation, and for the depths explored in the present study, the obvious lack of homogeneity of the head tissues does not prevent the use of an algorithm derived from work on homogeneous models to derive an accurate (within 1 mm) average estimate of the depth of the brain events responsible for the EROS effects.

In conclusion, the data of the present study suggest an approach for deriving tridimensional information about EROS effects within the brain which goes beyond the two-dimensional descriptions available so far. The approach indicates that, at least under some conditions (e.g., depth < 30 mm) and averaging across subjects, the depth of EROS effects can be localized within a few millimeters. Under these conditions, this accuracy is not very different from that obtained with standard fMRI procedures. In addition, EROS also provides information about the timing of cortical activity, with a resolution that rivals that of electrophysiological methods. The combination of spatial and temporal information makes EROS a promising tool for the study of the time course of activity in localized cortical areas, at least for depths of less than 3 cm.

ACKNOWLEDGMENTS

We thank Steve Pickup and Delvin Mellerup for their technical support for data collection. The research presented here was supported in part by Grant MH-57125 from the NIMH to Gabriele Gratton and by McDonnell–Pew Grant 97-32 to Monica Fabiani.

REFERENCES


