The event-related optical signal (EROS) in visual cortex: Replicability, consistency, localization, and resolution

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Abstract
We previously reported a fast and localized noninvasive event-related optical signal (EROS) elicited by visual field quadrant stimulation in occipital brain areas (Gratton, Corballis, Cho, Fabiani, & Hood, 1995). We now present a replication and extension of that experiment. In addition, we propose a new method for estimating the cross-validity of the results based on intersubject correlations, report further data on the localization of EROS, and present an analysis of its spatial resolution. The results replicate our original findings. The intersubject correlation is generally quite high (r = .6–.8). The spatial resolution analysis indicates that activities from cortical areas located approximately 1.5 cm apart are measurable independently without cross talk. This study provides further support for the idea that noninvasive optical imaging can be used to derive images of brain activity combining good spatial and temporal resolution.

Descriptors: Noninvasive optical imaging, Brain imaging, Event-related optical signal, EROS, Frequency-domain optical measures

Experiments on isolated neurons (Cohen, 1972) and hippocampal slices (Frostig, Lieke, Tsv, & Grinvald, 1990), as well as in vivo studies with implanted optical fibers (Rector, Poe, Kristensen, & Harper, 1997) have demonstrated that neuronal activity is accompanied by concurrent changes in light scattering. In an extensive series of studies, we have shown that a fast optical signal, probably due to scattering changes, can also be measured noninvasively in humans from the surface of the head (for reviews, see Gratton & Fabiani, 1998, 2001, 2002). This signal (the event-related optical signal or EROS) can be useful for brain imaging in cognitive neuroscience because it can yield functional images of neuronal activity in the human cortex with a good combination of spatial and temporal resolution (Barinaga, 1997; Gratton & Fabiani, 1998; Villringer & Chance, 1997). In the paper in which EROS was first described (Gratton, Corballis, Cho, Fabiani, & Hood, 1995), we reported a fast optical signal with a latency of approximately 100 ms in occipital areas in response to the stimulation of different quadrants of the visual field (obtained by means of a 2-Hz vertical grid reversal). In this article, we present a replication and extension of these original findings, based on more data points and a larger sample of subjects. The data provide information about the localization power, spatial resolution, consistency, and replicability of this method.

As mentioned above, since our original paper (Gratton, Corballis et al., 1995), we have run several additional studies (involving a total of more than 60 subjects) in which an EROS response was obtained in visual cortex with a latency between 60 and 120 ms (Fabiani, Ho, Stinard, & Gratton, 2003; Goodman-Wood, Bauer, Corballis, Hood, & Gratton, 1996; Gratton, 1997; Gratton, Fabiani, Goodman-Wood, & DeSoto, 1998; Gratton, Goodman-Wood, & Fabiani, 2001; Gratton, Sarno, Maclin, Corballis, & Fabiani, 2000; Hackley et al., 2000). In these studies, stimuli varied along a number of dimensions including stimulus type (e.g., alternating and flashing grids, geometric forms, or letters), frequency of stimulation (from 0.5 to 10 Hz), stimulus characteristics (e.g., color, intensity, spatial frequency, brightness, size), interstimulus intervals (fixed vs. variable), and experimental conditions (e.g., eye-dominance, attention manipulations, previous exposure). The amplitude and latency of the EROS response appears to be modulated by factors such as stimulation frequency, attention, and previous
exposure to the stimuli. The increase in the mean photon transit time corresponded to a delay in the transit of photon density waves through active areas that ranged (across studies) between 0.01 and 0.20° of phase (using a 110-MHz light modulation frequency), corresponding roughly to increases of the photon time of flight of about 0.25–5.0 ps. EROS responses, with different latencies depending on the experimental conditions, were also recorded from temporal cortex in the case of auditory stimuli (Rinne et al., 1999) and from motor cortex in the case of finger movements (DeSoto, Fabiani, Geary, & Gratton, 2001). In all these cases the latency of the EROS response was similar to that of electrophysiological measures recorded from the same subjects in the same experimental conditions.

Other laboratories have since reported the observation of fast optical signals, presumably of neuronal origin, in the somatosensory modality (Steinbrink et al., 2000) and motor modalities (Wolf et al., 2000). In the visual modality, we are aware of two such replication attempts. The first (Wolf et al., 2000) was carried out using a small sample size and somewhat different stimulation and measurement apparatus from those used in our original study. This study produced results that were consistent with our original observations (i.e., changes in both the amount of photons migrating through occipital areas as well as their time of flight, with a latency of approximately 100 ms from visual stimulation). A second study (Syre et al., 2000) was conducted in Berlin, and failed to report a consistent delay in photon delay after stimulation. However, this study did not include any systematic mapping of occipital areas, nor contralateral control of stimulated areas or averaging across subjects.

The purpose of the current study is to replicate our original study, based on a 2-Hz stimulation of different quadrants of the visual field, in order to determine whether our original observations are indeed reliable. The following changes were made from the original study:

a. A larger (8 subjects instead of 3), independent sample size was used.

b. A multichannel recording system was used (instead of the one-channel system used for the 1995 study), which allowed for the simultaneous recording from eight different source-detector pairs per session (in fact, 32 interleaved locations were used over four consecutive recording sessions). The 32 recording locations (identified by the midpoints of the source-detector pairs) were spaced symmetrically around a virtual point located on the midline, approximately 2 cm above the inion. They formed four horizontal rows with individual recording locations spaced 5 mm vertically and 15 mm horizontally.

c. Specific a priori hypotheses were made for the latency (100 ms) and localization (based on a contralateral inverted representation of the visual field) of the responses. Thus, the latency and the recording locations at which the responses were expected to be found were determined a priori and were the same for all subjects. In all cases, the locations corresponding to the other (nonstimulated) quadrants were used as controls, so that the same locations were used in turn as experimental and control conditions. These predictions were based on our previous studies. This rigorous approach was used to eliminate the possibility that capitalization on chance might affect the results.

d. A faster sampling rate (50 Hz rather than 20 Hz) was used.

e. 750-nm laser diodes (rather than a 715-nm LED) were used as sources. The change of wavelength was dictated by a change in instrumentation. However, scattering (fast) effects are supposed to be relatively wavelength independent (see Frostig et al., 1990).

All other aspect of stimulation and recording were identical to those used in the Gratton, Corballis, et al. (1995) study. In addition to the replication and extension aspects of this study, the current article includes a new method for determining the intersubjects consistency of the data and for the detection of outliers. This method is based on the computation of the correlation of the waveform describing the EROS time course of each subject with that of all the other subjects combined. We also report an initial analysis of the spatial spread (point-spread function) of the EROS responses. This analysis can be used to provide a conservative estimate of the spatial resolution of EROS.

Methods

Participants

Eight volunteers (4 women, age 19–25) participated in a six-session experiment after reading and signing informed consent forms. The experiment included four optical sessions (in which optical data were recorded) and two electrophysiological sessions [in which visual evoked potential (VEP) data were recorded]. For reasons of space, in this article we report data from the optical sessions only.

Stimuli and Procedures

Each session included 64 blocks of trials, each lasting 20 s, with 40 s of rest between blocks. The participants sat in a dimly illuminated room in front of a computer display located at a distance of approximately 55 cm. Each block began with the appearance of four stationary black-and-white vertical grids (spatial frequency = 0.25 cycles/°), on the four quadrants of the screen (centered 2.5° to the left and right and 2.5° above and below of a central fixation cross) and subtending a visual angle of 4° horizontally and 4° vertically. After 4 s, the bars of one of the four grids began reversing color. The reversal frequency was 2 Hz (a reversal every 500 ms). The grid reversal (stimulation) period lasted for 16 s, and was followed by 40 s of blank screen. There were 16 blocks for each quadrant stimulation condition. The quadrant stimulation conditions were intermixed following a Latin-square pattern, which was presented in reverse order to half the subjects.

Optical Recording

The optical data were recorded using a frequency-domain method, based on a multichannel Omnia® Tissue Oxymeter manufactured by ISS Inc. (Champaign, IL). The light sources consisted of laser diodes emitting light at 750 nm (near-infrared range), with a power of 1 mW. The current powering the laser diodes was modulated at 110 MHz. The light emitted by the laser diodes was channeled to the surface of the scalp through 0.4-mm-diameter optic fibers, that were held in place using a modified motorbike helmet. The detectors were two 3-mm-diameter fiber optic bundles connected to photomultipliers tubes (PMTs). The current feeding into the PMTs was modulated at 110.005 MHz (i.e., 5 kHz off with respect to the current modulating the laser diodes used as sources). This generated a 5-kHz heterodyning
channels with a standard error exceeding 0.8 and participant were substituted with the mean value. Also, standard deviations from the mean (‘‘spikes’’) for that channel points for which the value of the phase data was more than two (i.e., noise level) of each recording channel. Individual data across trials, to account for variations in the amount of light (and optical signal (EROS). The EROS activity was computed by the phase delay data were used for the computation of the ‘‘fast’’
described by Gratton and Corballis (1995).

The recording system allowed for the concurrent measurement of optical parameters from multiple channels (in this case, 8 at a time). During the four optical sessions, data were obtained from a total of 32 channels per participant, covering an area approximately 12 cm wide and 2 cm high. This afforded a spatial sampling of approximately 1.5 cm along the left–right axis and 0.5 cm along the top–bottom axis (with data referred to the midpoint between the source and the detector). The center of the recording area was located approximately 2 cm above the inion. Source-detector distances were between 2.5 and 3.5 cm, which, based on our previous work (Gratton, Fabiani, et al., 1995; Gratton et al., 2000), correspond to activity in brain areas at depths between 1 and 2 cm from the surface of the scalp. The 16 medial locations of this recording array can be expected to cover a large portion of the average surface projection of areas 17 and 18 (between 1 and 3 cm above the inion, up to 2.5 cm lateral on each side of the midline; Maier, Dagnelie, Spekreijse, & van Dijk, 1987) as well as surrounding cortical areas. The 16 lateral locations (8 on each side) can be expected to cover extrastriate occipital areas. Because two detectors were used simultaneously, distinguishing the signals from 8 different channels required multiplexing the sources. We used an electronic multiplexer that kept each source ‘‘on’’ for 5 ms, and ‘‘off’’ for 15 ms, in turn, thus yielding a 50-Hz effective sampling rate for each channel.

The stimulation and recording systems were synchronized using a parallel cable. The recording of the optical data began simultaneously with the beginning of each block, marked by the appearance of the four grids on the participant’s monitor, and continued for 20 s (thus providing 1,000 data points). The synchronicity between stimulation and recording was checked by additional recordings in which the recording fiber was placed directly over the computer screen used for stimulation. The pulse artifact on the optical data was compensated using an algorithm described by Gratton and Corballis (1995).

Data Analysis The phase delay data were used for the computation of the ‘‘fast’’ optical signal (EROS). The EROS activity was computed by segmenting the 16-s stimulation period into shorter epochs corresponding to each stimulation (i.e., grid reversal), with periods beginning 40 ms before a reversal. These segments were then averaged separately for each channel, quadrant stimulation condition, and participant, and the effects were standardized across trials, to account for variations in the amount of light (and therefore noise level) of each recording channel. Individual data points for which the value of the phase data was more than two standard deviations from the mean (‘‘spikes’’) for that channel and participant were substituted with the mean value. Also, channels with a standard error exceeding 0.8° were not included in the analysis (this led to discarding less than 10% of the data). A baseline comprising the last 40 ms (three data points) before each grid reversal was subtracted from each trial record. To reduce high frequency noise, an 8-Hz low-pass filter was applied to the data (see Maclin, Gratton, & Fabiani, 2003); to eliminate slow drifts the data were linearly detrended before the analysis.

The data were then analyzed using a computer program (Gratton, 2000) that allows the user to produce maps of the optical response for each data point, and overlay them over sample images of the surface of the cortex. For each data point, the maps are computed by back-projecting the value recorded at each channel (i.e., source-detector pair) on the cortical surface. In many cases, the regions investigated by different channels overlapped partially. In other words, the same cortical points were often investigated by several channels. In these cases, the values assigned to those points were derived by averaging the values of all these different channels (see pi-detector, Wolf et al., 2000, for a similar logic). The same program also allows for deriving statistical analyses (means, standard errors, and t scores) across subjects for each data point or for preset intervals and for plotting the data according to Talairach coordinates. Note that in the present case, the y (front–back) coordinate is arbitrary because the data are surface-projected, and only the x (left–right) and z (top–bottom) coordinates are relevant.

Two types of analyses were conducted, one related to the time course of the activity, and the other related to the spatial localization of the peak of the activity. The time course analysis was conducted by selecting a priori cortical locations corresponding to the four quadrants of the visual field. The following four sets of Talairach coordinates (in millimeters) were used: for the upper left recording point (lower right stimulation), x = −15, z = 0; for the upper right recording point (lower left stimulation), x = 15, z = 0; for the lower left recording point (upper right stimulation), x = −15, z = −15; for the lower right recording point (upper left stimulation), x = 15, z = −15. The coordinates of all four points correspond to area 17 in the Talairach database. For each subject and stimulation condition, the recording quadrant opposite to the stimulated field (e.g., the lower left recording quadrant in case of stimulation of the upper right visual field) was deemed as the predicted location, whereas the remaining three quadrants were used as control. Thus, the very same recording locations served, in turn, as predicted and control locations.

The peak activity analysis was carried out by selecting the peak with the maximum value in a specific region of interest. The left–right and top–bottom Talairach coordinates (in millimeters) of the margins of this region of interest were set to −15 to 15, and 0 to −20, respectively. Again, this area corresponds for the most part to area 17 in the Talairach database. This value was computed across subjects and separately for each subject. Note that, in the present study, there was no alignment of the data on the basis of individual differences in functional neuroanatomy.

Results The grand average EROS responses obtained from each of the locations selected a priori (upper left, upper right, lower left, and lower right) are shown in the different panels of Figure 1. In this figure, the EROS response for the stimulation condition for which a response was predicted at that particular location is presented separately from the average of the EROS responses for the other three control conditions (averaged together). For each of the four locations, the stimulation condition that was expected to produce a response (predicted condition) did, in fact, elicit activity with a latency of approximately 100 ms (on average, 116 ms), whereas no consistent response was observed at this latency for the other stimulation conditions (control condition). There was, in fact, a significant difference between the latency of the peak responses in upper areas (92 ms) and lower areas.
(140 ms), $t(6) = 2.827$, $p < .05$ (excluding Subject 6—see outlier analysis reported below). A possible interpretation of this difference is that, given the a priori nature of the analysis (which precluded alignment based on individual variations in functional anatomy) the lower regions included both striate and extrastriate areas in at least some participants. Also, for each location, the predicted condition also elicited a secondary response with a latency of approximately 350 ms.

Figure 2 (left panel) shows the grand average EROS response from the predicted (i.e., locations where a response was predicted to occur for each visual stimulation quadrant) and control (i.e., the locations where a response was predicted to occur when another visual quadrant was stimulated) conditions, averaged across the four selected locations. The grand average responses obtained in the Gratton, Corballis, et al. (1995) study are also reported here for comparison (right panel). The time course of the EROS responses (for both experimental and control conditions) obtained in the two studies are quite similar. In both cases, the EROS response in the experimental condition peaks at a latency of 100 ms and shows a secondary peak of minor amplitude at a latency of approximately 300–400 ms.

**Peak Analysis**

The amplitude of the EROS response at a latency of 100 ms after stimulation (compared to the prestimulus baseline value) for the predicted and control conditions for each individual subject is presented in Figure 3. The data in this figure are averaged across the four selected locations. The 8 subjects are ordered according to the size of their EROS response from the largest (leftmost) to the smallest (rightmost). Note that for all subjects except one the

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**Figure 1.** Time course of the fast optical response (EROS) observed at each of the four a priori selected locations for the predicted stimulation condition (open circles) and for each of the other control stimulation conditions (closed circles).

**Figure 2.** Left panel: Time course of the fast optical response (EROS) observed at the predicted (thick line) and control (thin line) locations in the current experiment. The error bars indicate the standard error of estimate computed across subjects. Right panel: Time course of the fast optical response (EROS) for locations with maximum response (thick line) and, from the same locations, when the opposite visual field quadrant was stimulated (thin line), in the Gratton, Corballis, et al. (1995) paper.
predicted condition elicited a larger response (characterized by an increase in phase) than the control conditions; this difference was significant, \( t(7) = 2.34, p < .05 \) (one-tailed). Simple effect analyses revealed that a response was observed for the predicted condition, \( t(7) = 3.04, p < .01 \) (one-tailed), but not in the control conditions, \( t(7) < 1 \). In fact, as noted by Gratton et al. (1997), the control locations showed a smaller response with a slightly longer response latency (approximately 160 ms), \( t(7) = 2.44, p < .05 \) (one-tailed). This may reflect the callosal transfer of the visual information from one hemisphere to the other, or intrahemispheric transfer from one area to another.

**Consistency Analysis**

These data indicate that, on average, there was a good replication of the data reported by Gratton, Corballis et al. (1995; see also Gratton et al., 1997). In the present study, we were also interested in addressing further issues related to the use of EROS. Specifically, (a) how consistent was the time course of the EROS response in the selected locations for each individual subject, and (b) is it possible to detect outliers whose waveform is substantially different from that of all other subjects. A third question, related to the "point spread function" (i.e., the size of the area where a presumably localized response can be recorded) of the EROS response, will be discussed in the next section of this article.

The time course of the EROS response for the stimulus predicted to produce the largest response (averaged across all four predicted locations) for each subject is presented in Figure 4. The data in this figure are presented in a standardized format (i.e., each subject’s values were divided by the standard deviation computed across time) to make the results more easily comparable. As in Figure 3, the subjects are ordered according to the amplitude of their EROS response. Note that 7 out of 8 subjects showed quite a similar time course—with the notable exception of 1 subject (Subject 6, white waveform).

To quantify this visual impression we used a measure of consistency obtained by computing, for each subject, the correlation of the EROS waveform with that obtained from averaging together the data of all the other subjects, according to the following formula:

\[
(i) = \frac{\sum (w(i, t)^* w(a - i, t))}{\sqrt{\sum w(i, t)^2 \sum w(a - i, t)^2}}
\]

where \( r(i) \) is the consistency index for subject \( i \), \( w(i, t) \) is the amplitude of the EROS response of subject \( i \) at time \( t \), and \( w(a - i, t) \) is the average amplitude of the EROS response of all other subjects at time \( t \). The consistency index (which is essentially an index of the correlation between an individual subject’s waveform and the average waveform of all the other subjects) can range between \(+1\) and \(-1\). Note that the correlation is computed across the whole waveform, and therefore does not reflect the selection of a particular data point. Because the correlation is computed between a subject and all the others, in the absence of a fast signal, the expected value for the average consistency index across subjects should be 0; conversely, a value consistently above 0 for different subjects indicates the presence of a fast optical response. The average consistency index computed across subjects (derived by first computing the Fisher transform of each subject’s consistency index, averaging these values, and then computing the inverse Fisher transform of the average value) is therefore a reflection of the “cross-validity” of the EROS waveform across subjects. A statistical test of the consistency index can be obtained by performing a \( t \) test of a difference from 0 of the Fisher-transformed data. Confidence intervals for the consistency index can also be computed according to the same logic.

The consistency index can also be used to “screen” individual subjects, and to determine whether an outlier is present in the data. This can be achieved by computing a “z-score” for each subject’s Fisher-transformed data. If this z score exceeds a preset criterion (such as a \( z > 1.96 \) in absolute value, corresponding to \( p < .05 \)), the waveform for that particular subject can be considered an outlier.

The consistency indices computed for each subject are reported in Table 1, separately for the predicted condition (averaged across selected locations), control condition (all other stimulation conditions for each selected location), and difference between predicted and control condition. The statistical analysis was conducted by calculating the Fisher transform of each consistency index, and computing the average, standard error, and \( t \) score (testing the hypothesis of an average value greater than 0) of the
transformed values across subjects. The averages and standard errors of the consistency indices are also reported in Table 1. The data reported in Table 1 indicate that the time course of the EROS response was consistent across all subjects but 1 (Subject 6), for whom the consistency index was negative. The z score associated with the Fisher transform of the consistency index of Subject 6 (based on the mean and standard deviation of this transform computed on all 8 subjects) was −2.16 for the predicted condition, and −2.05 for the difference between predicted and control conditions. Both values are beyond the .05 p level indicating that this subject can be considered an “outlier” with respect to the time course of the optical response at the predicted location. In fact, all other subjects are homogenous, with an average consistency index above 0.7. This suggests that (a) EROS data are generally consistent across subjects, and (b) subjects whose EROS data depart from the norm can be identified by computing their consistency with the rest of the subjects. This procedure can be used to detect outliers and improve the robustness of the results. The analyses presented in the remainder of this article will be based on 7 subjects, and Subject 6 (the outlier subject) will be excluded. Although outliers may occur for several different reasons, a potential problem in this study is that individual subjects’ data were not aligned according the individual differences in subjects’ functional neuroanatomy, which can be expected to be quite significant.

Localization of the EROS Response
Maps of the 100-ms EROS response observed for each quadrant-stimulation condition (averaged across 7 subjects—Subject 6 is

<table>
<thead>
<tr>
<th>Subject</th>
<th>Predicted</th>
<th>Others</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.66</td>
<td>0.79</td>
<td>0.27</td>
</tr>
<tr>
<td>S2</td>
<td>0.49</td>
<td>0.29</td>
<td>0.52</td>
</tr>
<tr>
<td>S3</td>
<td>0.92</td>
<td>0.74</td>
<td>0.88</td>
</tr>
<tr>
<td>S4</td>
<td>0.58</td>
<td>0.46</td>
<td>0.16</td>
</tr>
<tr>
<td>S5</td>
<td>0.77</td>
<td>0.13</td>
<td>0.71</td>
</tr>
<tr>
<td>S6</td>
<td>−0.72</td>
<td>0.72</td>
<td>−0.87</td>
</tr>
<tr>
<td>S7</td>
<td>0.90</td>
<td>−0.35</td>
<td>0.93</td>
</tr>
<tr>
<td>S8</td>
<td>0.84</td>
<td>−0.31</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Fisher avg. 0.67 0.38 0.54
Std. error 0.27 0.20 0.33
t(7) 2.89 1.99 1.80
p (one-tailed) 0.011 0.044 0.058
Avg. w/o S6 0.81 0.25 0.72
t(6) 6.05 1.49 4.64
p (one-tailed) 0.000 0.090 0.001

Figure 5. T-score maps of the fast optical response (EROS) at a latency of 100 ms for each quadrant stimulation condition. The area in darker gray represents the area explored by the measurement. The green box indicates the medial occipital area where the contralateral-inverted response was expected (“region of interest” for the analysis). The blue crosses within each box represent the locations of peak response for each condition. TL: top left quadrant stimulation condition; TR: top right quadrant stimulation condition; BL: bottom left quadrant stimulation condition; BR: bottom right quadrant stimulation condition.
not included) are presented in Figure 5. The location of the peak of activity for each of the four conditions is also reported in this figure (crosses), together with the region-of-interest for the study (box). This region of interest is related to the surface projection of primary visual cortex (area 17). On the basis of a contralateral inverted representation of the visual field in visual cortex, we expect the maximum of activity to be located in the opposite quadrant of this box (i.e., the peak of activity for each condition should be in an eccentric position with respect to the box). Figure 5 shows that the peak of the medial occipital EROS response is located just as predicted in all four conditions (i.e., in opposite quadrant of the region of interest). The probability of this occurring by chance is equal to 1/4 for each of the four conditions, which means, overall, 1/4, or 1/256, equivalent to a

\[ p < .005 \]

This suggests that EROS provides a significant localization of the activity, with errors smaller than the size of each quadrant of the region of interest. In fact, the location of the peak response is close to the “predicted location” for each experimental condition hypothesized earlier in the article and used for the a priori analyses presented above. The average distance (across conditions) between predicted and observed locations of the peak response is about 5 mm, which is roughly equivalent to the spatial sampling used in the study. Note that the plotting program used to derive Figure 5 includes an approximate alignment of the data averaged across subjects onto Talairach coordinates. This allowed us to determine that, on average, the areas of peak response at 100 ms latency were within area 17 (primary visual cortex, or V1). This finding is also consistent with our previous reports (Gratton, Corballis, et al., 1995; Gratton et al., 1997).

The maps reported in Figure 5 also show activity in areas outside of the region of interest. Because these areas of activation were not “predicted” a priori (partly because of the lack of previous studies) we will not discuss them further in the present article.

**Spatial Resolution of EROS Data**

The data presented in Figure 5 indicate that EROS has a good localization power, with errors typically within 5 mm (equivalent to the spatial sampling used in the study). However, a distinction needs to be made between spatial localization and spatial resolution. The first term refers to the ability to correctly identify the locus of the peak of activity, whereas **spatial resolution** refers to the ability to distinguish between two simultaneously active areas located in close proximity to each other. This latter property is closely related to the issue of cross talk between areas, that is, to how certain one can be that measurements taken from one particular location are not contaminated by activity observed at a different location. The spatial resolution of a technique is in large part determined by the point-spread function of the technique. With this term, we indicate how diffuse the measured response is in case of a supposedly very circumscribed focus of cortical activity. Different brain imaging methods differ greatly in terms of their spatial resolution. For instance, electroencephalographic methods (EEG, visual evoked potentials, and event-related potentials) possess a low level of spatial resolution because activity from any cortical source can spread to the whole scalp. This low spatial resolution can occur even though these methods may, at least in some cases, provide data with very good localization power. The maps presented in Figure 5 do not directly address the question of the spatial resolution of EROS data.

To provide initial data about this issue, we computed for each stimulation condition the amplitude of the EROS activity at a latency of 100 ms at a number of different locations within the region of interest. The locations were chosen so as to form a grid 30 mm × 15 mm wide, sampled every 5 mm. The four corners of the grid corresponded to the four locations where we expected the maximum response for each stimulation condition, according to the contralateral, inverted model of the primary visual cortex described earlier in this article. We then ordered the data from different locations as a function of their distance from the location predicted to have the maximum response (one of the corners of the grid, determined by the stimulation condition). The measurement locations were then grouped according to their distance from the predicted location in 4-mm bins, and averaged together (\( N \) was between three and five locations per bin). The resulting function is presented in Figure 6. In this figure, the amplitude of the EROS response is presented as a function of the distance of the measurement location from the predicted location of maximum response. This can be considered as a conservative estimate of the point-spread function of the optical response. Note that, in order to interpret the data presented in Figure 6 as a point-spread function of the EROS response, the following assumptions must be met: (a) the predicted locations correspond to the point of maximum cortical activity, (b) these points are the same for all subjects, (c) cortical activity only occurs in one point and does not extend across the scalp, and (d) the spatial sampling is sufficiently high so as to provide detailed information about the point-spread function. It is unlikely that all four of these assumptions will be met in this case; nevertheless, we can expect that violations of these assumptions will all lead to an increased “spreading” of the observed response, thereby making the estimate of the point-spread function even more conservative. The data from this figure indicate that (a) the maximum EROS response occurs at the predicted location (distance 0), (b) the EROS response is less than 50% at a measurement location 5 mm away from the predicted location (corresponding to a full-width, half amplitude estimate of less than 1 cm), and (c) the EROS response is practically absent (less than 15% of the maximum value) at distances above 15 mm. This suggests that little or no cross talk occurs at these distances, and that, therefore, activities occurring at cortical locations spaced 15 mm from each other can be recorded independently with little or no interference.
Discussion

The results of this study replicate and extend the data we reported in the Gratton, Corballis, et al. (1995) paper. As in the data reported in that study, an increase in photon delay (phase parameter) was observed with a latency of approximately 100 ms from quadrant stimulation. This response was localized. For any particular location where an EROS response was predicted to occur, the effect was only observable when the appropriate quadrant of the visual field was stimulated, but not when other quadrants were stimulated. From the point-spread analysis reported in Figure 6 we can also conclude that the effect did not spread more than 1.5 cm away from the active location.

The experiment was designed so that a number of control conditions were present. By varying the stimulation quadrant and the recording location at which the response was expected to occur in a systematic fashion, we eliminated the possibility that the effect was due to a generalized variation in light associated with the stimulus, to specific factors related to a particular source-detector pair, to movement or muscle artifacts, or to other aspects of the measurement apparatus. In addition, differently from our 1995 study, for each stimulation condition we determined a priori the locations at which we expected an effect (predicted locations) and those at which we expected no effect (control locations). Note that each recorded location served in turn both as a predicted and as a control location depending on the stimulation condition used. Given individual differences in the functional anatomy of the occipital cortex, this approach is likely to reduce the power of the experiment. However, this strictly hypothesis-driven approach was considered necessary to make certain that no optimization procedure was involved (and therefore no capitalization on alpha error occurred).

In addition to replicating the basic observation of a 100-ms EROS response, the data presented here also replicated the longer latency response observed in our previous study. This lends credibility to the hypothesis that this secondary increase in phase delay is a "real" phenomenon. However, the exact nature of this phenomenon is yet unclear. There are at least two explanations for this secondary increase in phase delay: (a) it reflects the occurrence of long-latency neuronal activity in occipital cortex (e.g., feedback activation from some other cortical area), or (b) it reflects the occurrence of some other physiological event which is time-locked but lagged with respect to the neuronal activity (e.g., glial activity related to potassium re-uptake or some metabolic phenomenon).

A question that arises is why there is a discrepancy between the conclusions drawn from the present study (as well as those from Wolf et al., 2000, and from our previous studies) and those drawn by the study reported by Syre et al. (2000). In that study, the authors claim that no significant effect was obtained in their attempt at replicating the Gratton, Corballis et al. (1995) paper. In addition, they claim that neuronal optical phenomena of the type reported by Rector et al. (1997) produce optical effects that are unlikely to be observable at the scalp with current methodologies.

To provide an explanation for these conflicting conclusions, it might be useful to make the following considerations. First, there were some differences between the study by Syre et al. (2000) and our current and previous studies. For instance, while our recordings were based on a systematic grid of locations, the work reported by Syre et al. was based on a series of sample locations that varied across subjects and did not systematically cover the medial occipital cortex. Because our data suggest that the optical response is highly localized, the procedure used by Syre et al. did not maximize the probability that a response could be detected if one did in fact exist (i.e., the power of the study). Second, differently from ours, the paper by Syre et al. did not report a hypothesis-testing statistical analysis computed across subjects. Indeed, some of the data reported by the authors show small increases in phase delay that occurred at a latency between 60 and 150 ms after stimulation, and that were apparently not inconsistent with the data we reported in the Gratton, Corballis et al. (1995) paper (although smaller in amplitude). Third, the stimulation condition used in the Syre et al. study were different than those used by Gratton et al. and in the present study, in that a "full-field" checkerboard was used, instead of a quadrant stimulation manipulation. As a result, it was not possible to construct the same types of control conditions that were used in the current study (i.e., to study the response at a particular location when different quadrants of the visual field were stimulated). As we mentioned earlier, the quadrant manipulation allows us to distinguish between specific effects of local brain activity and nonspecific phenomena due to the stimulation and/or recording apparatus. In conclusion, it is not clear that there is in fact a contradiction between the Syre et al. results and those presented in the current article (and in our other EROS studies). Substantial stimulation, recording, and analysis differences may account for the different conclusions reached by the authors. We believe that the current study provides a more systematic examination of the issue than that reported in the Syre et al. study.

It should be noted that the effects we obtained in the current study, although qualitatively similar to those reported in our previous paper (Gratton, Corballis et al., 1995), are much smaller in amplitude (approximately 0.03° of phase compared to about 0.2° of phase obtained in our previous study). This discrepancy may be due to one of three factors (or a combination of them). First, in the current study the locations at which the EROS effect was measured were fixed a priori and kept the same for each subject and condition, whereas in our 1995 paper we identified, for each condition, the location with the maximum response. This optimization procedure can be expected to result in much larger effects than those obtained when fixed locations are chosen. The "fixed-location" approach should be expected to have inherently less sensitivity and smaller signal-to-noise ratio than the optimization method. However, in the current study it was deemed necessary to provide a conservative approach in the detection of fast optical effects. Note that our estimates of the point-spread function of the EROS data suggest that even small variations (of the order of 5–10 mm) in the location of the peaks of activity in different subjects and condition may result in substantial reduction in the size of the observed effects. Although it is difficult to exactly assess the impact of this problem on the estimation of the amplitude of the EROS effects, we believe that a reduction of the effects by a factor of 2 or 3 can easily be the outcome of lack of alignment of the effects across subjects and conditions. Second, the wavelength used in the current study (750 nm) was slightly longer than that used in our original study (715 nm). This difference may result in a slight reduction of sensitivity to the scattering phenomena that are expected to subsume the fast optical effects. Third, the effect obtained in our original experiment was based on only 3 subjects—and therefore the confidence interval for our measurement was quite large. Indeed, the results obtained in our study are within the 90%
confidence interval that could be obtained from our original study. In conclusion, there is no obvious contradiction between the results of the current study and those reported in Gratton et al.

The current study provides further information with respect to our previous EROS studies. First, we report a new procedure for determining the cross-validity of EROS data and for detecting outliers. This procedure, based on the measurement of the correlation of the waveforms of a particular subject with the average waveforms of all other subjects, indicates that EROS waveforms are quite consistent ($r = .7–0.8$) across subjects, at least for the condition obtained in the current study. The consistency is to some extent a reflection of the signal-to-noise ratio: If the signal is very small or the noise very large, we should expect variable and inconsistent waveforms across subjects. Thus, consistency could be improved by a better alignment of the activity for different subjects and conditions. Methodology for such an alignment (based on the digitization of the recording locations, alignment on structural magnetic resonance images, and three-dimensional reconstruction of optical data) are being developed in our laboratory and are being used for analysis of new data sets (that now include structural MR and digitizing of recording locations for all subjects). These alignment procedures can be expected to lead to significant improvements in the signal-to-noise ratio and consistency of the EROS data. Other important factors in noise reduction are the use of appropriate filtering methods, stronger or more effective light sources, and the use of an appropriate number of trials in the recording. The number of trials used in the current study was relatively large, but it should be noted that, because a relatively small number of channels (eight) were recorded during each session, four sessions had to be run to collect enough trials for the study. This limitation may be overcome by the use of more powerful machines capable of recording from a much larger number of channels at once.

A second set of novel data obtained in the current study refers to the spatial resolution of EROS. This was estimated by measuring the amplitude of the EROS response at various distances from the location where the effect was expected to be maximal. This analysis indicated that the amplitude of the EROS response drops quite rapidly with distance, so that it is only half as big at distances of 5 mm from the expected peak location, and less than 20% at distances of 15 mm or more. This should be considered as a conservative estimate of the actual point-spread function of the optical response, as many factors that could be expected to increase the “blurring” of the signal were not controlled in the current study (including variations in the locations of activity in different subjects and conditions, lack of appropriate alignment, lack of a well-developed three-dimensional reconstruction algorithm, reduced spatial sampling). Notwithstanding all these limitations, the spatial resolution of the EROS signal is still good and not very different from that of more developed noninvasive brain imaging methods (such as fMRI), at least for cortical locations. Several investigators have suggested that noninvasive optical methods, being based on a fundamentally random diffusion process, may have limited spatial resolution. Although this may appear to contrast with our previous statement, the following should be noted:

a. The term limited applied to spatial resolution is relative and needs to be specified further. In fact, it is clear that the spatial resolution of noninvasive optical imaging methods is to be measured on the order of millimeters or centimeters and not microns as is the case for optical studies of exposed cortex. Indeed, our study represents one of the very first efforts to provide a quantitative estimate of the spatial resolution of noninvasive optical methods.

b. Our measurements are based on the “phase” parameter, rather than on the intensity measures used in most optical imaging studies. Theoretical speculations lead us to hypothesize that phase measures should be more localized than intensity measures. This has both positive and negative consequences. On the one hand, the greater localization of the signal should lead to an increased spatial resolution and specificity. On the other hand, a localized signal is inherently not very sensitive, as effects can be detected only if the measurements are taken from the correct locations. Our data suggest that it is sufficient to move the detector 1 cm away from the correct location to observe a reduction in the signal by a factor of 5 or 10. This means that a high spatial sampling (1 cm or less) is required for appropriate measurement of EROS data.

The data in the present study (as well as those in all our other studies) indicate that brain activity results in an increase in the delay of photon waves passing through the relevant cortical area. Single-unit studies, however, often report a reduction in scattering associated with neuronal activity (e.g., Cohen, 1972). How can a reduction in scattering lead to an increase in phase delay? In homogeneous media, a decrement in scattering over the entire medium should result in a reduction of the phase delay. However, the situation is very different when the scattering decrement is localized to a relatively deep layer (1–2 cm from the surface of the head). The result of a decrement in scattering in a deep layer is that photons can penetrate somewhat further into the tissue, rather than being reflected (back-scattered) toward the surface by the scattering layer itself. As a result, the balance of photons reaching different depths is shifted, and a larger proportion of photons will tend to follow a longer path before reaching the detector (a 1-ps delay corresponds to about a 200-μm path lengthening). The end result is therefore an increase in the phase delay averaged across all photons that reach the detector. These phenomena (illustrated in Figure 7) were clearly shown in Monte Carlo simulations we presented in an earlier paper (Gratton, Fabiani, et al., 1995). The relative reduction of back-scattering of the cortex may also be the cause of the reduction in light also concurrent with brain activity [shown in human studies by Steinbrink et al., 2000, and replicated by Maclin et al., 2003 (this issue), and in animal work by Rector et al., 1997]. In fact, a decrement in scattering with activity resulting in a reduced amount of light reaching the detector and mirroring the time course of electrical activity has been consistently reported in in vivo animal work (see Rector, Harper & George, 2002, for a review). However, this intensity reduction effect is in part counteracted by the increased transparency of the tissue. Therefore, the intensity effects may be more variable and in general smaller than the phase delay effects.

In conclusion, the present study reports a replication of our original observations about the possibility of detecting noninvasively a fast and localized optical response with a latency of approximately 100 ms after the stimulation of one quadrant of the visual field. For each visual field quadrant that is stimulated, the signal occurred in a different location of the occipital cortex in a manner predictable on the basis of the contralateral inverted representation of the visual field in primary visual cortex. The
response was localized so that any particular location was activated only when a particular visual field quadrant was stimulated but not when the other quadrants were stimulated.

These responses are consistent with our previous studies and with independent work conducted at the University of Illinois (Wolf et al., 2000).

REFERENCES


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