Effects of measurement method, wavelength, and source-detector distance on the fast optical signal

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Received 27 October 2005; revised 9 March 2006; accepted 16 May 2006
Available online 26 July 2006

Fast optical signals can be used to study the time course of neuronal activity in localized cortical areas. The first report of such signals [Gratton, G., Corballis, P. M., Cho, E., Fabiani, M., Hood, D., 1995a. Shades of gray matter: Noninvasive optical images of human brain responses during visual stimulation. Psychophysiol, 32, 505–509.] was based on photon delay measures. Subsequently, other laboratories have also measured fast optical signals, but a debate still exists about how these signals are generated and optimally recorded. Here we report data from a visual stimulation paradigm in which different parameters (continuous: DC intensity; modulated: AC intensity and photon delay), wavelengths (shorter and longer than the hemoglobin isosbestic point), and source-detector distances (shorter and longer than 22.5 mm) were used to record fast signals. Results indicate that a localized fast signal (peak latency = 80 ms) can be detected with both delay and AC intensity measures in visual cortex, but not with unmodulated DC measures. This is likely due to the fact that differential measures (delay and AC intensity) are less sensitive to superficial noise sources, which heavily influence DC intensity. The fast effect had similar sign at wavelengths shorter and longer than the hemoglobin isosbestic point, consistent with light scattering but not rapid deoxygenation accounts of this phenomenon. Finally, the fast signal was only measured at source-detector distances greater than 22.5 mm, consistent with the intracranial origin of the signal, and providing indications about the minimum distance for recording. These data address some of the open questions in the field and provide indications about the optimal recording methods for fast optical signals.

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Keywords: Fast optical signals; Event-related optical signal (EROS); Scattering; Optical brain imaging

Introduction

Fast optical signals provide a tool for imaging human brain function with sub-cm spatial resolution and ms-level temporal resolution. Ten years ago, we reported the first non-invasive measurement of fast changes in the optical properties of the human brain in response to visual stimulation (Gratton et al., 1995a). Due to limitations in the recording apparatus, this initial study was carried out on a very small subject sample (N = 3) and was based on a relatively slow sampling rate (20 Hz). The data were obtained with a frequency–domain optical spectrometer. The phase delay measures in response to the stimulus showed a few picoseconds increase in the mean time taken by photons to move between sources and detectors located on the surface of the head, compared to average baseline measures obtained immediately before the stimulus. We labeled these optical changes the “event-related optical signal” or EROS, as they are obtained by observing the average optical brain response time locked to a stimulus or other event.

This initial observation was followed by a series of studies replicating these effects in the visual domain with larger sample sizes (Gratton, 1997; Gratton et al., 1998, 2000, 2001; Gratton and Fabiani, 2003). Additional studies showed that fast optical signals can be obtained with other modalities and from other cortical areas, including auditory (Rinne et al., 1999; Tse et al., 2006; Tse and Penney, in press; Fabiani et al., 2006), somatosensory (Maclin et al., 2004), motor (Gratton et al., 1995b; DeSoto et al., 2001), and frontal cortices (Low et al., 2006).

All these studies provided results that were consistent with the original finding, showing increases in delay in active cortical areas. They also indicated that the optical changes are simultaneous with electrical changes measured with evoked potentials (EPs; Gratton et al., 1997, 2001; Maclin et al., 2004) or event-related brain potentials (ERPs; DeSoto et al., 2001; Fabiani et al., 2006; Low et al., 2006). Finally, comparisons of the localizations of optical responses with changes in the blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) signal also indicated substantial correspondence between the two sets of responses (Gratton et al., 1997, 2000).
Other laboratories (Wolf et al., 2000, 2002, 2003a,b; Tse and Penney, in press) have recently reported similar fast optical effects (rapid increases in phase delay in association with cortical activation) in the visual, motor, and auditory modalities, all obtained with frequency-domain methods.\(^1\) Two other laboratories have also reported the occurrence of fast optical signals but have used different recording methods in which reductions in the intensity of light reaching the detectors was the main finding rather than increases in photon delay (Franceschini and Boas, 2004; Steinbrink et al., 2000). Intensity changes were also observed by Wolf et al. (2003a,b), concurrently with the phase delay effects.

In parallel with the recordings obtained in humans, Rector et al. (1997; see also Rector et al., 2005) demonstrated that fast optical changes, presumably due to changes in light scattering, can also be observed in freely behaving animals. This finding is consistent with earlier reports obtained in isolated neurons (e.g., Cohen et al., 1971) and hippocampal slices (Frostig et al., 1990).

These data demonstrate the feasibility of detecting fast brain responses, presumably directly related to neuronal activity, using optical methods. Such data can potentially be very useful in cognitive neuroscience research because they provide a good combination of spatial and temporal information and thus may facilitate our understanding of cortical dynamics. However, questions remain about which measurements – changes in intensity or delay – are optimal for recording fast optical signals. A direct comparison based on the extant literature is difficult because only a few studies have reported both intensity and delay effects recorded from the same subjects in the same study (Wolf et al., 2003a,b; Maclin et al., 2003).\(^2\) Further, the results of these studies have revealed some inconsistencies, with some showing more evident results with intensity measures and others with delay measures. Intensity measures are simpler and less expensive to obtain. However, continuous unmodulated (DC) intensity measures may be sensitive to noise from many different sources, including superficial events (such as capillary pulse) and environmental light sources extraneous to the measurements. In contrast, measures that are based on variations over time in the amount (AC intensity) as well as the velocity (delay) of the photon diffusion process may be exquisitely sensitive to differential effects occurring deep into the tissue and provide better control for external sources of noise.

The current paper is aimed at providing a more systematic examination of the parameters for the non-invasive recording of fast optical signals. The methodology used, based on frequency–domain optical methods (Gratton et al., 1990), allows for the direct comparison of intensity and delay changes during visual stimulation. We used a larger subjects sample than those used in our previous studies (\(N = 19\)), and recorded from a dense array of channels (160), all located over occipital areas, thus providing a high spatial sampling. In addition to comparing intensity and delay data, we also examined the effects of two wavelengths (690 and 830 nm) and the effects of source-detector distances in the recording montage.

The use of multiple wavelengths provides some information about the biophysical phenomena underlying the fast optical signal. Specifically, two hypotheses about the generation of fast optical effects can be contrasted: the scattering hypothesis and the rapid deoxygenation hypothesis. Fast optical effects are commonly considered to be due to rapid scattering changes co-occurring with neuronal activity (presumably due either to changes in membrane transparency or to movement of water from intra- to extra-cellular compartments during neuronal depolarization). Whereas considerable evidence for this interpretation is available in in vitro tissue preparations (e.g., Frostig et al., 1990), no direct demonstration of the scattering hypothesis has been presented in vivo, and in particular from the intact human head. A possible alternative explanation is that the fast optical effects recorded at the scalp in humans may be due to rapid deoxygenation of the hemoglobin contained in brain tissue such as those reported with high field functional magnetic resonance imaging (Hu et al., 1997), and with exposed cortex optical imaging in animals (Vanzetta and Grinvald, 1999). According to this hypothesis, the occurrence of cortical activity should induce a rapid increase in oxygen consumption in a particular cortical area (see also Weber et al., 2004). When this occurs, hemoglobin becomes more deoxygenated. These two hypotheses can be contrasted by determining whether fast optical imaging effects have the same or different sign (e.g., increases in photon delay) at wavelengths shorter and longer than the hemoglobin isosbestic point (800 nm—the point at which the absorption spectra of oxy- and deoxy-hemoglobin cross over). If rapid deoxygenation is the cause of the fast optical signal, it should invert sign when measured using wavelengths on opposite sides of the hemoglobin isosbestic point, as an increase in the concentration of deoxy-hemoglobin should be associated with a concurrent decrease in the concentration of oxy-hemoglobin. If instead the fast optical signal is due to changes in scattering, similar results should be observed for both wavelengths (although presumably effects should be slightly larger in absolute terms at shorter wavelengths, as scattering decreases with the fourth power of the light wavelength). Thus, a comparison of the fast optical effects at 690 and 830 nm should distinguish between these two possibilities.

A third hypothesis is that the fast optical signal is related to rapid changes in blood flow or blood volume. However, empirical evidence for such changes is currently scanty: Although reports exist of changes of this type within 1 s from stimulation (e.g., Lindauer et al., 1993; Jones et al., 2001), their time course (onset latency longer than 0.5 s) is slower than that of the fast optical signals as reported in the literature. For this reason, this hypothesis will not be further considered here.

This study also provides empirical data assessing the optimal range of source-detector distances to be used for recording the fast signal to maximize the exploration of brain volumes rather than of more superficial structures (such as muscles or skin).\(^3\) The relative

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\(^1\) One lab did report inability to obtain reliable phase delay changes with visual stimulation, and presented Monte Carlo simulation data purportedly indicating that the phase delay changes should be too small to be detected using current technologies (Syré et al., 2003). Notably, the spatial sampling used in that study was much inferior and less systematic than that used in the work from our laboratory, and this report has in fact been followed by a number of positive findings when the measurements have been taken with methods more closely matching those used in our lab.

\(^2\) Frequency domain equipment is needed to obtain such data, as continuous wave systems cannot record photon delay measures but are limited to light intensity.

\(^3\) Evidence that the fast signals are generated inside the brain has been presented in several studies (e.g., Gratton et al., 1995a,b, 1997, 2000), which have shown close correspondence between the locations at which such signals are observed and the cortical areas activated by a particular stimulus (as determined by fMRI).
depth of the area probed by the optical sensors can be investigated by varying the source-detector distance (e.g., Gratton et al., 2000; Choi et al., 2004). At very short source-detector distances (less than 20–25 mm), the volume investigated by non-invasive optical methods should be very superficial (at most 1 cm deep), including only very small portions of brain tissue. However, at longer source-detector distances this volume should encompass a greater portion of brain tissue. Thus, as fast optical signals are generated inside the brain, we should expect these signals to increase in size with source-detector distance. Conversely, noise signals generated in the skin, superficial muscles, or the environment should remain constant or decrease in amplitude with source-detector distance. However, with very long source-detector distances (exceeding 40–50 mm), relatively little light is expected to reach the detector and the signals can become noisy and unstable. Thus, it is important to establish the optimal range of source-detector distances for the recording of optical signals and to exclude distances that would contribute data from structures other than the brain and/or add to the noise. Because the optical montage used in the current study used a variety of source-detector distances for the same surface locations, it is possible to estimate the size of the fast optical effect as a function of source-detector distance and thus assess the minimum usable distance for fast optical recording.

As in our previous optical imaging work on visual cortex (Gratton et al., 1995a,b; Gratton and Fabiani, 2003), we used a pattern-reversing stimulus. This type of stimulus was chosen because it is isoluminant and minimizes stimulus-related changes in environmental light. This study also used a number of recent methodological innovations designed to improve spatial resolution and increase the power and rigor of the statistical analyses, which are described in more detail in the Methods section. This study was also part of a larger project investigating neurovascular coupling, whose results are beyond the scope of the current paper and will be reported elsewhere. However, for comparison and validation purposes we also report here visual evoked potentials (VEPs) that were recorded concurrently with the optical data. The experimental design and the use of multiple wavelengths also allowed us to compute slow/hemodynamic optical effects, which are also reported here in a summary fashion for comparison with the fast signal.

**Methods**

**Participants**

Nineteen young adults (9 females, age 20–28, mean age 22.3) participated in a multiple-session study, including an MR session (during which structural MRs were collected) and an optical session (during which both optical imaging and ERP data were collected). All subjects reported themselves in good health and free from medications affecting the central nervous system. All subjects had normal or corrected to normal vision.

The data were collected within the context of a larger project investigating neurovascular coupling in younger and older adults. For the purpose of the current paper, only a subset of the data are presented. All participants signed written consent forms and were compensated for their time. All aspects of the experiment were approved by the local IRB and conformed to the Helsinki declaration for the protection of human subjects.

**Task and stimuli**

During the optical recording, participants sat in front of a computer monitor centered at eye-level at a distance of approximately 80 cm. The optical recording was divided into two sessions, with data from a different set of 80 overlaid optical channels (defined by a particular source-detector pair) collected from each. A set of 80 channels is referred to as a “montage”. A session consisted of 60 blocks, each lasting 60 s. Every block began with a 20-s period during which a fixation cross was displayed. After the initial fixation, a black and white checkerboard appeared and the cells switched color (i.e., reversed), with a frequency that varied in different blocks. The checkerboard subtended a total visual angle of 15° horizontally and 17° vertically and had a spatial frequency of 0.4 cycles/degree.

The reversal frequencies used were 1.0417 Hz (1-Hz condition), 2.0833 Hz (2-Hz condition), 4.1667 Hz (4-Hz condition), 6.25 Hz (6-Hz condition), and 8.3333 Hz (8-Hz condition). The reason for these stimulation frequencies was that they are all (apart from 8.33 Hz) factors of the sampling rate (62.5 Hz), thus an integer number of sampling points occurred between stimuli. The reversal frequency conditions were presented with the following order (once for each montage): 1-Hz, 2-Hz, 4-Hz, 6-Hz, 8-Hz, 1-Hz, 2-Hz, 4-Hz, 2-Hz, 1-Hz, 8-Hz, 6-Hz, 4-Hz, 2-Hz, 1-Hz. An unequal number of blocks for the different frequencies was used to reduce the difference between the number of reversals (trials) across conditions by increasing the number of blocks at the lowest frequencies.

Each block started with 20 s of fixation, followed by a stimulation period lasting 19.2 s, and was followed by a 20.8-s blank period. For the purposes of the current paper, only data from the reversal period are considered, with each reversal considered as a stimulus. The total amount of light generated by the computer screen was identical during all phases of the stimulation, and did not vary when a reversal occurred, as the color reversal of each square leads to an isoluminant condition. The only environmental light variation was determined by the refreshing rate of the computer screen (70 Hz), which, however, was faster than retinal persistence and not time locked with the stimulation conditions across stimuli, blocks, or subjects. The participants’ task was to fixate at the center of the checkerboard. Brief rest periods were given every 5 blocks.

**Optical recording**

Optical data were recorded using an Imagent device (ISS Inc., Champaign, IL), based on 64 laser diode sources (32 emitting light at 690 nm and 32 emitting light at 830 nm) and 8 photomultiplier tubes (PMTs) as detectors. Only a subset of the source laser diodes were used at one time, using the montage described in Fig. 1. The light sources reached the head by using 0.4-mm optic fibers, whereas 3-mm fiber optic bundles were used for connecting the head to the detectors (photomultiplier tubes, PMTs). Source and detector fibers were held in place on the participant’s head using modified motorcycle helmets. Hair was combed away from under the detector fibers before they were put in place; the source fibers, being much smaller, could be placed through the hair. The use of a rigid helmet minimized the effects of head and body movements on the recordings. Source fibers were paired, so that each location was illuminated by a fiber connected to a 690-nm laser and another connected to an 830-nm laser (which were never turned on at the same time).
The light sources were time multiplexed in cycles of 16 ms divided into ten 1.6-ms periods (1.6 ms “on” periods, and 14.4 ms “off” periods for each source), so that at any given time each detector could only receive light from one source closer than 6-cm distance. Our measurements indicate that the amount of light transmitted between a source and a detector at distances exceeding 6 cm is negligible (Gratton and Fabiani, 2003).

The light sources were modulated in intensity at 110 MHz. The average intensity during the “on” periods was less than 10 mW per source; considering the off periods, each source emitted an average of less than 1 mW light power, well below FDA standards. The detector amplifiers were modulated at a frequency of 110.00625 MHz. This created a cross-correlation (also called heterodyning or beating) frequency of 6.25 kHz. This frequency was used for the recording of frequency–domain data. The optical data from each detector were sampled continuously at a frequency of 50 kHz. Eight points were collected for each oscillation of the cross-correlation frequency. As each source was on for 1.6 ms, there were 10 oscillations per source during each multiplex period. To eliminate the possibility of crosstalk between sources, we discarded the first two oscillations (.32 ms) of recording from each channel. The remaining eight oscillations (for a total of 64 digitized points) were used to compute a fast Fourier transform (FFT) yielding estimates of DC intensity (zero-frequency or average effects), AC intensity (amplitude of oscillations at the 6.25-kHz cross-correlation frequency), and phase delay4 for each source/detector combination (80 per montage, 160 in total) with an effective sampling period of 16 ms (62.5 Hz).

**Electrophysiological recordings and analysis**

VEPs were recorded continuously during the stimulation period at 200 Hz from eight scalp electrodes (Fz, Cz, Pz, T5, T6, P3, P4, and right mastoid) referred to the left mastoid using a 1- to 100-Hz bandpass, with a 60-Hz notch filter. The data were transformed off-line into an average mastoid reference system. Vertical and horizontal electro-oculograms (EOG) were recorded bipolarly from electrodes placed above and below the right eye and to the left and right of the outer canthus of each eye, respectively. EOG artifacts were corrected off-line using a procedure described by Gratton et al. (1983). Data were segmented into epochs time locked to each stimulus, starting with a 25-ms pre-stimulus baseline. Trials with shifts exceeding 200 μV were discarded. Separate averages were computed for each subject, electrode, and stimulation condition. Note that the optical and electrophysiological data were recorded simultaneously.

**Structural MRI**

All subjects included in this study underwent a high-resolution structural MRI scan in a Siemens 3-T Magnetom Allegra MR Headscanner located at the Biomagnetic Imaging Center (BIC) of the University of Illinois at Urbana-Champaign. Using an MPRAGE sequence, a 144-slice scan with a 1.2-mm slice thickness was obtained either in the sagittal or axial plane. The pulse parameters used in MR recording included a repetition time of 800 ms, an echo time of 4.38 ms, and a flip angle of 8°. The field of view was 240 × 240 × 172.8 mm with matrix dimensions of 192 × 256 × 144 and voxel size of 1.25 × 0.938 × 1.2 mm.

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4 In a heterodyning system, a change in phase at the beating frequency reflects an equivalent phase change between the input frequencies. As the beating frequency is always lower than the original frequencies, this represents an amplification of the time difference between the original frequencies.
the source and detector positions on the MR anatomical image obtained for each participant (see above), allowed us to co-register subjects co-registration of visual cortex. Fifth, two-dimensional structural MR images. This allowed for a more accurate between-between the left and right calcarine fissures as identified in the individual anatomical images were centered on the midpoint for the influence of anatomical variability across participants, the source and detector points were subjected to Talairach transformation.

### Table 1

<table>
<thead>
<tr>
<th>Steps in the analysis of fast optical data</th>
<th>Pre-processing</th>
<th>Mapping and statistical analysis</th>
<th>Co-registration of optical and MR anatomical data</th>
</tr>
</thead>
<tbody>
<tr>
<td>For each channel, condition, and participant</td>
<td></td>
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<td></td>
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<tr>
<td>Phase wrapping correction and transformation in picoseconds</td>
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<tr>
<td>Correction of slow drifts</td>
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<tr>
<td>Pulse correction</td>
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<tr>
<td>DC and AC normalization</td>
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<tr>
<td>Computation of modulation</td>
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<td></td>
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<tr>
<td>Filtering (optional)</td>
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<td></td>
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<tr>
<td>Estimation of phase SD (to discard noisy channels)</td>
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<tr>
<td>Segmentation into stimulus (or response)-locked epochs and averaging of like trial types</td>
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<tr>
<td>Filtering (optional)</td>
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<tr>
<td>Baseline subtraction</td>
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</table>

### Table 2

<table>
<thead>
<tr>
<th>Estimated signal-to-noise (S/N) at various stages in the analysis</th>
<th>No. of trials</th>
<th>No. of channels</th>
<th>Frequencies (Hz)</th>
<th>No. of subjects</th>
<th>Signal (ps)</th>
<th>Noise (ps)</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data collection</td>
<td>1</td>
<td>1</td>
<td>31</td>
<td>1</td>
<td>1.0</td>
<td>133.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Averaging</td>
<td>240</td>
<td>1</td>
<td>31</td>
<td>1</td>
<td>1.0</td>
<td>8.6</td>
<td>0.116</td>
</tr>
<tr>
<td>Spatial reconstruction</td>
<td>240</td>
<td>6</td>
<td>31</td>
<td>1</td>
<td>1.0</td>
<td>3.5</td>
<td>0.285</td>
</tr>
<tr>
<td>Frequency filtering</td>
<td>240</td>
<td>6</td>
<td>10</td>
<td>1</td>
<td>1.0</td>
<td>1.1</td>
<td>0.882</td>
</tr>
<tr>
<td>Grand average</td>
<td>240</td>
<td>6</td>
<td>10</td>
<td>19</td>
<td>1.0</td>
<td>0.3</td>
<td>3.846</td>
</tr>
</tbody>
</table>

The steps of the analysis of fast optical data are presented schematically in Table 1. To provide a reference point, we presented the estimated signal-to-noise achieved at each major stage of the analysis in Table 2. The signal size estimates were obtained from the grand average effects reported in this study, whereas the noise estimates were obtained from the standard deviations of the single trial data. Note that such estimates are heavily dependent on the conditions used in a particular experiment and therefore may be hard to generalize across studies.

The optical data from each participant and location were first corrected for phase wrapping around the 360° mark and transformed into picoseconds. Then, slow drifts were corrected using a polynomial regression method (effectively eliminating frequencies below 0.01 Hz). A pulse correction algorithm developed by Gratton and Corballis (1995) was then applied. DC intensity and AC intensity were normalized by dividing them by the average value across each block. For each channel (i.e., source-detector combination), an estimate of the standard deviation (SD) of the phase was obtained and used to discard channels with excessive variability (SD > 160 ps), usually due to insufficient amount of light reaching the detectors to provide valid estimates. This procedure led to discarding approximately 35% of the channels, mostly because the montage comprised a large range of source-detector distances (including very long ones). Table 3 reports, for each source-detector distance, the total number of channels from which data were recorded, as well as the number of channels with acceptable noise levels.

The data were then segmented into 240 ms epochs, time locked to each pattern reversal (i.e., stimulus), beginning 16 ms before each reversal. The first five stimulations from each block were discarded to generate stable stimulation conditions. The remaining epochs were used to compute average waveforms for each channel and subject, and the results were averaged over a total of 240 trials in the 1-Hz condition, 560 trials in the 2-Hz condition, 900 trials in the 4-Hz condition, 920 trials in the 6-Hz condition, and 1240 trials in the 8-Hz condition. The data were then filtered to eliminate frequencies above 10 Hz (to increase signal-to-noise ratio, see Maclin et al., 2003), and a baseline (estimated as the average of a 32-ms peri-stimulus period) was subtracted from the data. This baseline was selected to reduce the impact of the previous trial while maintaining sufficient baseline duration.

For each participant, a 2.5-mm grid was established over the surface of the occipital scalp (centered on the mid-calcarine point,
as described above). For each location of the grid, the channels whose recording volumes encompassed the location were then determined (independently for each participant). For delay data, the different channels were then averaged together. For DC intensity, AC intensity, and modulation, the logarithms of the values were averaged, achieving the mathematical equivalent of a “pi detector” (Wolf et al., 2000). For phase data, the absolute value (rather than the logarithm) was used for the following reasons. First, phase delay may be negative. As a consequence, the product of two negative values would generate a positive number, which would be incorrect in this case (the sign of the effect would depend on the number of channels crossing a particular point). Second, whereas effects at different locations along the photon path combine in a multiplicative fashion for intensity measures, they combine in an additive fashion for phase delay measures. Note that the volumes corresponding to each channel (i.e., source-detector pair) were “discretised,” that is, any given pixel was classified as being either in or out of the path rather than being assigned a probability.

Most points in the central grid region were within the path of several (up to 20) channels that were weighted equally. This allowed us to select channels with specific source-detector distances—an important prerequisite for the analysis of source-detector distance effects. In fact, for most of the analyses (apart from that of the source-detector distance effect), only channels with source-detector distances between 22.5 mm and 50 mm were used. The rationale for this choice is that channels with source-detector distances shorter than 22.5 mm are unlikely to be sensitive to cortical activity (at least in occipital areas), and channels with source-detector distances exceeding 50 mm are typically very noisy.

For each measurement type, the previous methods yielded a grid (map) of activity for each time point (15 time points; sampling rate: 16 ms), stimulation condition, and participant. These maps were spatially filtered using an 8-mm Gaussian filter (2 cm kernel), and used for statistical analysis. This analysis was conducted by computing three types of score maps: one related to the difference between the average maps and the pre-stimulus baseline across stimulation conditions (average effect), one related to the linear trend between different stimulation conditions (with the 1-Hz stimulation condition expected to produce the largest response, and the 8-Hz stimulation condition expected to produce the smallest response), and one related to the 1-Hz condition compared to baseline. Each of these t score maps was converted into Z score maps. Following the methodology commonly used to correct for the problem of multiple comparisons when analyzing brain imaging data, the significance of a response was estimated by comparing the Z score value observed at the peak point with a criterion value estimated using a “blob analysis” approach (see Friston et al., 1995).

This method assumes that the spatial sampling is relatively homogenous over the area in which the criterion is computed. However, the montage used only afforded a relatively constant spatial sampling (i.e., channel density) in the central recording region, whereas spatial sampling was much sparser at the periphery of the recording region. Therefore, a region of interest (ROI) encompassing the central recording region was used for this estimation. The use of a restricted ROI also increased the power of the analysis. Expressed in Talairach coordinates, the ROI ranged from −21 to +21 along the x (right-left) axis and between −12 and +12 along the z (bottom-top) axis. Note that the ROI was not defined along the y (back-front) axis because only surface projections were used.

In order to reduce the number of comparisons and reduce the probability of alpha error, only the maps obtained at a latency of 80 ms from pattern-reversal stimulation were used for the determination of the statistical significance of an effect. This latency was selected on the basis of previous studies (e.g., Gratton et al., 2001). In all cases, directional hypotheses were made (with expected increases for the delay parameter, and decreases for all other parameters). Criteria corresponding to one-directional P values of 0.05 (corrected for multiple comparisons) were used to estimate the significance of effects.

The methods used in the current study (using multiple wavelengths and a blocked stimulation design) also permitted the computation of slow optical effects. Relative oxy- and deoxy-hemoglobin concentration changes were estimated using standard procedures (Boas et al., 2001) and averaged across blocks for each subject, stimulation condition, and source-detector pair.

Results

Overall analyses

The purpose of this section is to report the overall effects obtained in the study, in order to demonstrate that they were similar to those reported in previous work. As the current study was part of a larger project investigating the relationship between neuronal and vascular measures, a more complete analysis of the relationship between various measures will be presented elsewhere. Here we only present summary measures for VEPs and slow/hemodynamic optical effects for comparison and validation purposes.

A summary of the VEPs recorded in the current study is presented in Table 3. Following Gratton et al. (2001), we report the VEP in terms of variability (standard deviation) across electrodes for each stimulation frequency condition. Although this measure does not provide information about the electrode at which effects are largest, it provides useful information about the latency at which the electrodes separate from each other the most, indicating the occurrence of localized brain activity. Note that two peaks are observable in the variability waveforms (shaded gray in Fig. 2): one with a latency of approximately 80 ms and one with a latency of approximately 128 ms. They correspond roughly to the C1 and N1 components of the VEP, thought to correspond to activity in striate and extrastriate cortex, respectively. As in Gratton et al. (2001), both these components are much larger for the 1-Hz condition than for the other stimulation conditions, and, as

<table>
<thead>
<tr>
<th>Distance (mm)</th>
<th>Average number of channels recorded</th>
<th>Channels with acceptable noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5–17.5</td>
<td>14.1</td>
<td>14.1</td>
</tr>
<tr>
<td>17.5–22.5</td>
<td>44.1</td>
<td>42.3</td>
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<tr>
<td>22.5–27.5</td>
<td>33.8</td>
<td>27.6</td>
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<tr>
<td>27.5–32.5</td>
<td>24.3</td>
<td>15.7</td>
</tr>
<tr>
<td>32.5–37.5</td>
<td>15.8</td>
<td>5.8</td>
</tr>
<tr>
<td>37.5–42.5</td>
<td>10.4</td>
<td>1.1</td>
</tr>
<tr>
<td>42.5–50</td>
<td>9.3</td>
<td>0.2</td>
</tr>
<tr>
<td>&gt;50</td>
<td>8.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Total</td>
<td>160.0</td>
<td>107.2</td>
</tr>
</tbody>
</table>
expected, the amplitude of the response appears to be inversely related to stimulation frequency (e.g., Van der Tweel and Verduyn Lunel, 1965).

Fig. 3 reports statistical (Z scores computed across subjects) surface maps (back views) of the oxy- and deoxy-hemoglobin effects during each stimulation period (measured during the 10 and 20 s from stimulation, averaged across the 5 stimulation frequency conditions. Red and yellow indicate increases in hemoglobin concentration with respect to baseline (z > 2.0), and blue indicates decreases. The region of interest is indicated by the green box. Right column: time course of oxy- and deoxy-hemoglobin concentration changes at surface location x = 12, z = 0 (in Talairach coordinates).

Fig. 4. Grand average coronal (back view) maps of the peak latency of the fast optical response (delay left; AC intensity: right). The data were based on Z score maps computed across subjects at different latencies from stimulation. The color indicates the latency of the peak Z score value for that particular voxel (positive for delay and negative for AC intensity). In this figure, only voxels in which the Z score was greater than +2 for delay or smaller than −2 for AC intensity (at any latency) are shown. TL = top left; TR = top right; BL = bottom left; BR = bottom right.
interval between 10 and 20 s after the beginning of stimulation). Fig. 3 also reports the time course of the oxy- and deoxy-hemoglobin changes (right panel). For all these figures, data are collapsed across stimulation frequencies. These data indicate that the visual stimulation caused an increase in oxy-hemoglobin and a decrease in deoxy-hemoglobin, consistent with a typical blood flow (BOLD) effect. This effect occurred over an extended period of time (several seconds), also consistent with extant literature (e.g., Villringer and Chance, 1997).

Fig. 4 reports summary grand average surface maps of the fast optical response measured using the delay parameter (left) and AC intensity parameter (right). In Fig. 4, only voxels with a cross-subject Z exceeding the value of +2 (for delay) or lower than −2 (for AC intensity) are shown. The color indicates the latency of the peak response (the color scale indicating the actual latency of the response in ms is reported below the maps). The data are collapsed across stimulation frequencies and wavelengths. For the delay parameter, Fig. 4 indicates the occurrence of a medial response with a latency of approximately 80 ms, followed by a more lateral response with a latency of 120–160 ms. The latencies of these two responses correspond roughly with those of the two peaks of the VEP reported in Fig. 2. Further, the total area activated (albeit at different latencies) corresponds roughly with that showing a slow optical response in Fig. 3. These data suggest that the fast optical signal provides temporal information about the latency of activity in cortical areas. However, for the AC parameter, a response exceeding the criterion (with a latency of approximately 80 ms) could be observed only over the right occipital area.

Fig. 5 reports simplified tomographic reconstructions (for the principle used for estimating the depth of effects, see Gratton et al., 2000) of the fast optical effect at a latency of 80 ms for delay and AC intensity. The data are presented as axial Z score maps (computed across subjects), corresponding to the z = 0 slice in Talairach coordinates. Separate maps are presented for the average across conditions, the inverse linear trend as a function of stimulation frequency, and for the 1-Hz condition. Although preliminary, these data are consistent with the summary latency maps presented in Fig. 4, in that a bilateral, medial response is observed for the delay parameter, whereas the AC intensity parameter shows only a right hemisphere response. The linear trend (slope) data indicate that the 1-Hz condition shows a larger response than the other stimulation conditions (consistent with the VEP data).

Fig. 5. Axial grand average Z score maps (computed across subjects, Talairach coordinate of the slice: z = 0) of the fast optical response at a latency of 80 ms from stimulation for each contrast condition (average, slope, and 1-Hz condition alone) for delay (top row) and AC intensity (bottom row). The green box indicates the region of interest; the white cross indicates the peak point. Red and yellow colors indicate increases in the parameter with respect to baseline ($Z > 2.0$), and shades of blue indicate decreases in the parameter. Peak $Z$ = peak Z value within the region of interest (positive for delay, negative for AC intensity); Crit. = criterion Z score for statistical significance, taking into consideration the problem of multiple comparisons. FL = front left; FR = front right; BL = back left; BR = back right.
All of the data presented so far were obtained across subjects. The individual subject effects for the delay parameter are presented in Fig. 6. The graphs depict the mean amplitude of the response for each subject at a latency of 80 ms and taken at the peak location of the maximum effect for the group (Talairach coordinates: \(x = 12, y = \text{surface}, z = 0\)). One subject did not have valid channels at this location. In the right panel, the responses are presented in picoseconds. In the left panel, they are presented as a fraction of the variability (SD) measured on single trials for each channel (before filtering). Note that approximately half of the subjects showed a response exceeding 1 ps at this location, and approximately two thirds of the subjects showed a response exceeding 0.01 standard units.

Comparison of different measures of the fast signal

The delay parameter grand average Z score maps at a latency of 80 ms from the onset of the reversal, averaged across stimulation conditions (average), for the linear trend (slope) and for the 1-Hz condition (1 Hz), are presented in Fig. 7A. The Z score maps for AC intensity, modulation, and DC intensity are shown in Figs. 7B, C, and D, respectively. Data obtained with 690 nm and 830 nm light were combined for this analysis. These data indicate that a significant fast optical response can be observed in occipital cortex at a latency of 80 ms from stimulation (pattern reversal). As predicted, this response was characterized by an increase in delay and a reduction in AC intensity and modulation. The results for DC intensity were much less clear, and it is not obvious that a significant response could be observed with this method, once corrected for multiple-comparisons. The results of trend analysis (slope) indicate that the fast optical effects decrease with stimulus frequency, confirming our previous findings (Gratton et al., 2001), and in agreement with the electrophysiological data obtained in the current as well as in previous studies (Van der Tweel and Verduyn Lunel, 1965; Gratton et al., 2001).

For delay and AC intensity the grand average time course of the response at the peak location for each type of contrast is presented in Fig. 8. This figure illustrates the mean size of the response with each measurement technique. It indicates that, in the 1-Hz condition, the delay change is of the order of 2 ps, whereas the AC and modulation effects are of the order of 1 part over a thousand (the \(\log_{10}\) of the effect, multiplied by 100, is reported in Fig. 8—note that \(10^{0.04/100} \approx 1.001\)). All effects are 2–3 times larger in the 1-Hz condition alone than in the other contrasts, as expected based on the known physiology of electrical steady-state responses in visual cortex. The sizes of these effects are of the same order of magnitude as those reported in previous studies (e.g., Maclin et al., 2003).

Overall, these data imply that fast optical responses can be observed in a robust manner with different dependent variables (such as phase delay, modulation and AC intensity measures), although unmodulated DC intensity measures are less effective in measuring these signals.

Comparison of different wavelengths

Fig. 9 shows grand average time course data separated by source wavelength (690 and 830 nm) and measure (delay and AC intensity) for the 1-Hz condition at the Talairach location \(x = 12, z = 0\) (corresponding maps are shown in Fig. 10). The results show that there is a substantial similarity of the 1-Hz fast optical signals measured with 690 nm and 830 nm light. The AC response appeared slightly larger at 690 nm, but also noisier (as indicated by the larger error bars). For the AC intensity measures at 830 nm, differences begin to emerge later in the epoch, past the 80-ms peak of activity that is common to all graphs and corresponds to the latency of the earliest electrophysiological response. In fact, whereas the other three measures return to baseline by the end of the epoch, the AC measure at this wavelength remains relatively flat. This is likely to reflect the influence of slow oxygenation changes (in this case, increases in oxy-hemoglobin due to a “BOLD-type” response) superimposed on the fast effect. This shift may occur because the rise function of the BOLD response extends over a very long period of time (see Fig. 3). These effects are much less visible in the delay parameter, and for the AC measures taken at 690 nm.

In summary, these data are consistent with the hypothesis that scattering is the dominant factor underlying fast effects and are
Fig. 7. Grand average coronal (back view) $Z$ score maps of the fast optical response at a latency of 80 ms from stimulation for each contrast condition (left column = average, middle column = slope, and right column = 1-Hz condition) for delay (A), AC intensity (B), modulation (C), and DC intensity (D). Red and yellow colors indicate increases in the parameter with respect to baseline ($Z > 2.0$), and shades of blue indicate decreases in the parameter. Other data reported in the figure have the same meaning as in Fig. 5.
Fig. 8. Time course of the fast optical response at Talairach coordinates $x = 12$, $y = \text{posterior surface}$, $z = 0$, for the delay parameter (left panel, in picoseconds) and AC intensity parameter (right panel, expressed as $\log_{10} (\text{AC change}) \times 100$). Error bars represent the standard error computed across subjects, separately for each time point. The vertical bar at time zero indicates a pattern reversal in the checkerboard stimulus.

Fig. 9. Grand average time course of the fast optical response at the location of peak response for the 1-Hz condition, for delay (top row) and AC intensity (bottom row), and for 690-nm light (left column) and 830-nm light (right column). The data are from surface location $x = 12$, $z = 0$ (in Talairach coordinates). The delay is expressed in picoseconds, and the AC intensity as $\log_{10} (\text{AC change}) \times 100$. The error bars represent the standard error computed across subjects, separately for each time point.
clearly in contrast with the rapid deoxygenation hypothesis. Whereas the scattering hypothesis predicts same-sign effects at wavelengths placed at either side of the hemoglobin isosbestic point, the rapid deoxygenation hypothesis predicts effects of opposite sign.

**Effect of source-detector distance**

To study the effect of source-detector distance, we separately measured the amplitude of the fast response at the point of peak for channels grouped by source-detector distance. To obtain enough channels for reliable measures, we selected five 5-mm source-detector distance bins: 12.5–17.5 mm, 17.5–22.5 mm, 22.5–27.5 mm, 27–32.5 mm, and 32.5–37.5 mm. Data from both wavelengths (690 nm and 830 nm) were combined for this analysis. Only the 1-Hz condition data were used for this analysis because this condition exhibited the largest fast response and therefore was expected to be more stable even at long source-detector distances. To make the results from different distances comparable, we used the same surface location (Talairach coordinates: \(x = 12, z = 0\)) and selected channels crossing this point but varying in source-detector distance. Note that, due to the variability of the sensors placements with respect to the individual subjects’ brains and to the different amount of noise across subjects, not all subjects contributed to all bins. The amplitude of the fast signals for each bin is presented in Table 4, separately for phase delay and AC intensity. The same data are presented in graphic form in Fig. 11.

These data indicate that the fast optical response, measured either with delay or AC intensity measures, increases as a function of source-detector distance. The response is practically non-existent, or even of opposite sign (for the delay parameter) at short source-detector distances (less than 22.5 mm), and quite large at long source-detector distances (exceeding 22.5 mm). However, at the longest source-detector distances, the data became very noisy, as indicated by the large error bars in Fig. 11. When separated in 5-mm bins, only the delay effects between 22.5 and 32.5 mm distance were significant. These results are consistent

### Table 4

<table>
<thead>
<tr>
<th>Distance (mm)</th>
<th>Delay Signal</th>
<th>Delay Noise</th>
<th>AC intensity Signal</th>
<th>AC intensity Noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5–17.5</td>
<td>0.553</td>
<td>0.439</td>
<td>1.262</td>
<td>0.013</td>
</tr>
<tr>
<td>17.5–22.5</td>
<td>1.027</td>
<td>0.486</td>
<td>2.113</td>
<td>0.004</td>
</tr>
<tr>
<td>22.5–27.5</td>
<td>0.751</td>
<td>0.304</td>
<td>2.472</td>
<td>0.018</td>
</tr>
<tr>
<td>27.5–32.5</td>
<td>2.095</td>
<td>0.608</td>
<td>3.445</td>
<td>0.026</td>
</tr>
<tr>
<td>32.5–37.5</td>
<td>1.212</td>
<td>1.399</td>
<td>0.866</td>
<td>0.021</td>
</tr>
<tr>
<td>37.5–42.5</td>
<td>3.664</td>
<td>2.865</td>
<td>1.279</td>
<td>0.244</td>
</tr>
<tr>
<td>42.5–47.5</td>
<td>3.057</td>
<td>0.551</td>
<td>2.573</td>
<td>0.033</td>
</tr>
</tbody>
</table>

The distances 22.5–50 mm are those used for all the analyses presented in the study and are shown here for comparison purposes.
with predictions based on anatomical information, as channels with short source-detector distances are unlikely to reach the visual cortex and to be affected by brain phenomena, whereas channels with long source-detector distances are much more likely to reflect intracranial activity. In summary, these data are consistent with the brain origin of fast optical effects and indicate the minimum distances that would be useful for recording fast signals, at least from visual cortex.

Discussion

The results of the current study indicate that different dependent variables (i.e., phase delay, modulation, and AC intensity) can all be used to measure fast cortical phenomena, at least in visual cortex. The results of the wavelength analyses are consistent with the scattering hypothesis and are clearly inconsistent with the fast-deoxygenation hypothesis for the origin of the fast signal, although other hypotheses (such as rapid blood flow effects) were not directly tested. The distance analyses are consistent with the brain origin of the effects and suggest that care must be taken in choosing (a priori) or selecting (post hoc) the range of distances that can be used to best observe these effects.

The issue of the method of measurement of fast optical response deserves further discussion. First, consistent with previous findings, our current data indicate that phase delay, modulation, and AC intensity all can be used to measure fast optical responses, whereas similar clear-cut results are not obtained with unmodulated DC intensity measures. This appears to contrast with results from another laboratory that has instead reported fast optical effects with continuous-light measures (e.g., Steinbrink et al., 2000). However, we do not believe that these results are necessarily in contradiction. In fact, when measures are taken with unmodulated DC instruments, it is indispensable to eliminate any possible contamination from extraneous environmental light sources—which may occur in some cases and not others. This requirement is not as strict for AC measures, as external sources of noise can be distinguished from brain effects because the latter are carried by the modulation frequency whereas the former are not. Thus, we believe that the lack of a significant effect in our unmodulated DC data may be due to the greater difficulty in controlling the noise with these measures than with AC measures. Other labs may have applied a stricter control of these noise sources than we did (such as taking measurements in the dark, etc.). Note that although Franceschini and Boas (2004) reported their measures as intensity effects, they used a low-frequency modulation method (a few hundred Hz). Therefore, their measures should be considered more similar to the AC than the DC intensity measures reported in the current paper.

Given the significantly lower cost of instruments for intensity measurement than of those for the measurement of phase delay, the current results suggest that it may be a good economic strategy to focus on the former rather than the latter. However, such a conclusion may not be entirely appropriate. In fact, some of the results of the current study suggest that phase delay may actually be the measure of choice when the interest is in fast optical signals. First, the signal-to-noise ratio (as expressed by the Z score) is slightly larger for the phase signals. Second, AC intensity measures appear to be more contaminated by slow effects due to oxygenation changes than are the phase measures. Although both measures may be sensitive to fast and slow effects, it appears that phase measures are relatively more sensitive to fast effects and intensity measures are relatively more sensitive to slow effects. Further, whereas fast signals based on phase delay have been obtained from most areas of the brain (such as frontal, e.g., DeSoto et al., 2001; parietal, e.g., Maclin et al., 2004; temporal, e.g., Rinne et al., 1999; and occipital cortex, e.g., Gratton et al., 1995a,b), it remains to be established whether variations in the anatomy of activated areas will influence the effectiveness of intensity measures in revealing fast signals. For instance, Maclin et al. (2004) showed that a 16-ms response in the fast optical signal elicited by median nerve stimulation could be observed with delay but not with AC intensity measures, whereas a longer latency response to the same stimulation could be measured with both parameters. Although more research is needed to definitely confirm this hypothesis, it is possible that different types of instruments may be better tuned for the detection of fast and slow effects. Of course, multi-wavelength frequency–domain instrumentation does allow the recording and comparison of different signals in parallel and therefore provides more information than continuous wave instrumentation. This multi-measure approach would prove useful if it were shown that the various measures are not redundant, and are differently sensitive to the signal and/or to the noise. In the current study, it appears that delay and intensity parameters are differentially sensitive to contaminations.
It may at first appear surprising that the modulation signal is more robust than the DC signal. However, this occurs because the modulated light (AC) represents only a fraction of the total light (DC). Hence, a stimulus-related change in modulated light influences proportionally more the AC signal, and therefore the modulation, than the DC signal. For the same reason, noise influencing only the unmodulated portion of the light will also have comparably less effect on the modulation parameter. Thus, the modulation has a better S/N ratio than the unmodulated DC.

The results of the source-detector distance analysis indicate that fast optical effects are best recorded using source-detector distances exceeding 22.5 mm. This is consistent with the brain origin of these effects. However, they also indicate that at distances of 40–50 mm or more, the effects become very noisy and very few channels can be used, at least with the methodology used in the current study (although this number depends on the criterion used to accept a channel and on other parameters used in the recording). This provides some indication of the optimum source-detector distances to be used to record these effects. The data also suggest that the sign of the phase delay effect may actually depend on the source-detector distance: at very short source-detector distances the effect may actually invert. A bipolar effect of this type for the phase delay parameter is predicted by theory of the photon wave propagation (see Fishkin and Gratton, 1993) and by Monte Carlo simulations (see Gratton et al., 1995b). In simple terms, this phenomenon is due to the fact that the phase delay is related to the weighted average of the travel time of photons following different trajectories. Because of the physical arrangement used in our head measurements (in which sources and detectors are located on the same side of a bounded surface), photons traveling along superficial paths tend to follow shorter paths than photons traveling along deeper paths. Thus, the same scattering (or absorbing) object located at a given depth may intersect the path of relatively slow photons for short source-detector distances and of relatively fast photons for long source-detector distances. This should produce opposite-sign effects on the weighted average of the photon time-of-flight, which is essentially what is measured by the phase delay parameter. As a consequence, theory predicts a bimodal effect of phase delay as a function of source-detector distance. This is exactly what appears to occur in our study. However, when the effect occurs at sufficient depth with respect to the source-detector distance, the effect may occur consistently in the same direction (increases in phase for increases in transparency, or vice versa for reduction in transparency). Hence, for future studies, we advocate using distances of at least 22.5 mm.

In summary, the results of the current study indicate that fast optical effects due to scattering changes occurring in the brain can be measured non-invasively with different types of dependent variables, including phase delay, AC intensity, and modulation. They also show how refinement of the optical recording and analysis techniques can contribute significant advancements to the concurrent measurement of the localization and time course of neuronal signals.

Acknowledgments

The work presented in this paper was supported by NIBIB grant # EB002011 to G. Gratton and by NIA grant #AG21887 to M. Fabiani. We are grateful to Dr. Ed Maclin for comments on an earlier version of the manuscript.

References


Choi, J., Wolf, M., Toronov, V., Wolf, U., Polzonetti, C., Hueber, D., Safonova, L.P., Gupta, R., Michalos, A., Mantulin, W., Gratton,


Tse, C.-Y., Penney, T.B., in press. Optical imaging of cortical activity elicited by unattended temporal deviants. IEEE EMBB.


