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Functional Frequency-Domain Near-Infrared Spectroscopy Detects Fast Neuronal Signal in the Motor Cortex

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Millisecond changes in the optical properties of the human brain during stimulation were detected in five volunteers using noninvasive frequency-domain near-infrared spectroscopy. During a motor stimulation task we found highly significant signals, which were directly related to neuronal activity and exhibited much more localized patterns than the slow hemodynamic signals that are also detected by the near-infrared method. We considerably reduced the noise in the instrumental system and improved data analysis algorithms. With the achieved signal-to-noise ratio, single subject measurements were feasible without the requirement of particularly strong stimuli and within a reasonable period of measurement of 5 min at a mean signal-to-noise ratio of 3.6. The advantage of this noninvasive technique with respect to electrical recording is that it is able to detect neuronal activity with the relatively high spatial resolution of 8 mm. © 2002 Elsevier Science (USA)

INTRODUCTION

Brain activity is associated with physiological changes of the optical properties of the tissue that can be measured by noninvasive near-infrared spectroscopy (NIRS). Two major types of signals following brain stimulation can be distinguished. The first signal occurs in the range of seconds after the onset of the stimulation and reflects mainly changes in light absorption. The activated area of the brain consumes more oxygen and glucose, which leads to an increase in blood flow, which is accompanied by an increase in oxyhemoglobin concentration (O_2Hb) and a decrease in deoxyhemoglobin concentration (HHb), which are both detected by NIRS. This signal has been demonstrated by many authors (Maki *et al.,* 1995; Hirth *et al.,* 1996; Obrig *et al.,* 1996; Villringer and Chance, 1997, Colier *et al.,* 1999; Franceschini *et al.,* 2000; Toronov *et al.,* 2000, 2001) and corresponds to the BOLD signal, which is the basis of the fMRI technique.

The second signal consists of fast changes in the optical properties of cerebral tissue, which appear in the range of milliseconds after stimulation. These changes presumably are due to an alteration of the scattering properties of neuronal membranes (Gratton *et al.,* 1995, 1997a,b,c, 2000; Gratton and Fabiani, 2001; Rinne *et al.,* 1999; Steinbrink *et al.,* 2000; DeSoto *et al.,* 2001), which are simultaneous with electrical changes, cell swelling, and increased heat production (Tasaki, 1999). Thus the optical signal is directly related to neuronal activity, as in EEG or MEG, which is in contrast to conventional functional NIRS, fMRI (BOLD signal) or PET, which detect only the slow hemodynamic response to neuronal activity.

Compared to the slow hemodynamic signal the fast neuronal signal is difficult to detect, because the optical changes are small and other physiological signals, such as the hemodynamic pulsatility due to systole and diastole (Gratton and Corballis, 1995) and the cerebral vasomotion at 0.1 Hz (Mayhew *et al.,* 1996; Zheng *et al.,* 2001), dominate. Therefore, the system has to be highly noise optimized.

We have developed a low-noise instrument and data analysis algorithms to optimally detect the fast neuronal signal. The aim of this study was to use our system during functional motor activation by finger tapping, to ascertain that we can detect a functional fast signal and to characterize it.

Since both the slow hemodynamic and the fast neuronal signal can be detected by NIRS, we were able to compare the two with respect to colocation.

MATERIAL AND METHODS

Subjects

Two female and three male healthy adult volunteers (between 30 and 55 old) years were included in this study. They were all right-handed. Written informed consent was obtained from all subjects prior to the measurements.

NIRS Instrument

We used a frequency-domain tissue oximeter (Oxy-Imager, ISS Champaign, IL). For this instrument and for our application the largest source of noise is the shot noise, which results from the quantum nature of the light detection. Higher light intensity is the only way to improve the signal-to-noise ratio concerning the shot noise. The light of four
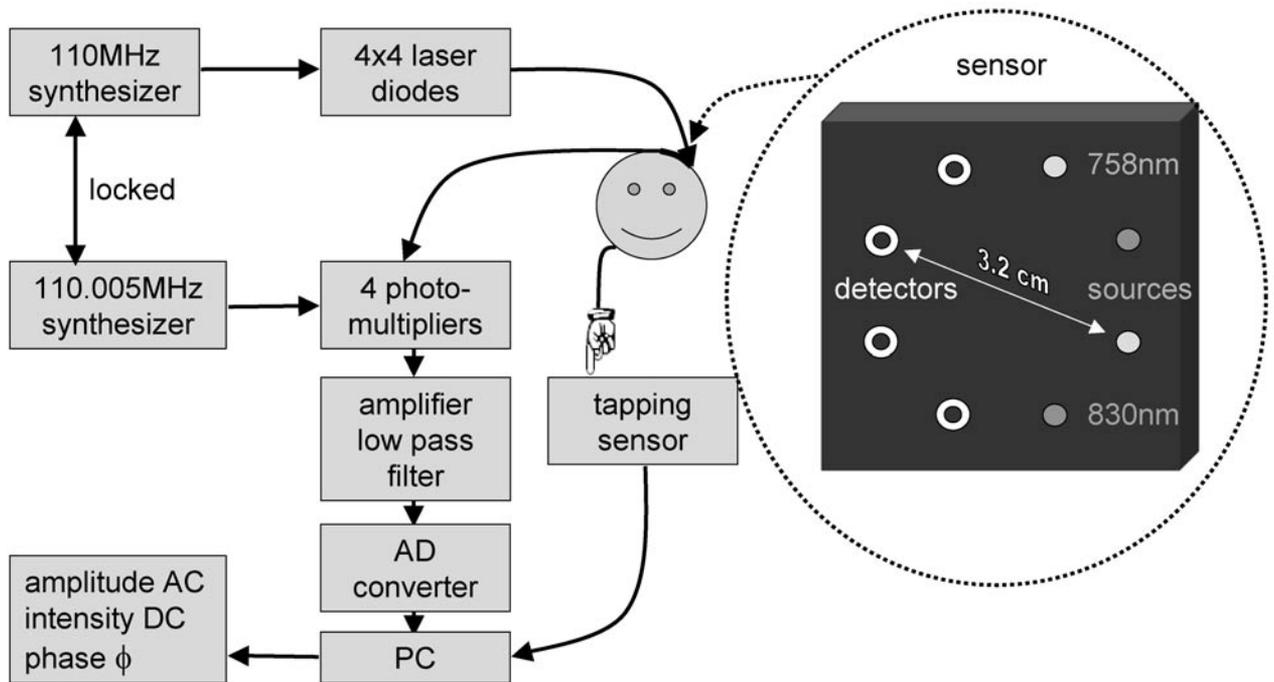
FIG. 1. Our frequency-domain near-infrared spectrophotometer (Oxy-Imager, ISS Champaign, IL) with a diagram of the sensor is shown.

laser diodes at either 758 or 830 nm was combined to achieve a higher light intensity at each of the four source locations (in total, 16 laser diodes). The light intensity of the laser diodes was modulated at 110 MHz and each quadruplet was synchronized to within 1° by tuned fiberoptics. To distinguish the light from the four source locations the laser diodes were multiplexed. The light was guided to the tissue by optical fibers of 400 μm core diameter. After passing through the tissue the light was collected by fibers of 3 mm core diameter. Four photomultiplier tubes were used as light detectors. The second dynode of each photomultiplier tube was modulated with 110.005 MHz to demodulate the high frequency. The resulting 5-kHz signal was low-pass filtered (cutoff, 10 kHz) and sampled by a 16-bit analog-to-digital (AD) converter. The following parameters were determined: modulation amplitude (AC), average value (DC), and phase (ϕ) of the intensity wave. The sample rate for a complete cycle, which measures all of these parameters for the four sources at each of the four detector locations, was 96 Hz.

The probe has four crossed source detector pairs arranged in a circle 3.2 cm in diameter (Fig. 1). The path of the light, which penetrates the tissue from a source to the detector, forms a banana shape, which is called a light bundle (Okada and Delpy, 2000). Source and detector can be swapped without affecting the shape of the light bundle. The geometry of the sensor was optimized to penetrate the region of interest with a high number of light bundles, both from a geometrical point of view and with regard to technical considerations, i.e., the detector has a limited dynamic range and therefore it is preferable to put all sources on one side and all detectors on the other side.

An electronic device registered the tapping movement. The amplitude of this tapping signal was independent of the amplitude and strength of the tapping. A pulse oximeter (N200, Nellcor Incorporated) measured the pulse wave and the arterial hemoglobin oxygen saturation (SaO_2) at the middle finger of the left hand, and a respiratory strain gauge (New Life Technologies) measured the respiration rate. All these signals were recorded simultaneously with the optical data.

Protocol

The probe was placed above the motor cortex (C3 position) according to the international 10/20 system (Jasper, 1957), contralateral to the hand performing the tapping exercise. By a metronome the tapping frequency was set at 2.5 times the subject's heart rate to avoid the influence of harmonics of the hemodynamic pulse due to systole and diastole on the detection of the fast neuronal signal. During the measurements, we recorded the heart rate reading from the pulse oximeter to ensure that the heart rate did not synchronize to the tapping frequency. For both conditions a stimulation period consisted of a sequence of alternating periods of 20 s of tapping and 20 s rest. The conditions were based on the following protocols. Condition 1, with one stimulation run: After 1 min of baseline measurement, there was a stimulation period of 5 to 10 min using the whole hand in the tapping, followed again by 1 min at baseline. Condition 2, consisting of four successive stimulation runs: After 1 min at baseline, there was a 5-min stimulation period with the index finger tapping against the thumb. Subsequently a 1-min baseline was taken followed by

a second 5-min stimulation period, where the middle finger tapped against the thumb. After an additional 1-min baseline a third 5-min stimulation period was taken with the fourth digit followed by another 1-min baseline and a fourth 5-min stimulation period with the fifth digit and a final 1-min baseline. The subject was seated in a quiet and dark room during the whole measurement. This study was approved by the Institutional Review Board of the University at Urbana-Champaign (IRB 94125).

Control data were obtained from a solid silicon block of approximately the same optical properties as the human head.

Data Analysis

Fast neuronal signal. To reduce physiological noise the arterial pulsatility was removed using an adaptive filter, which has previously been described in detail (Gratton and Corballis, 1995). In short, it extracts a mean shape of the pulse by screening each trace separately for pulses, whose period is adjusted before the averaging. This mean shape corresponds to the best estimate of each pulse and is used to remove each pulse from the data. This again requires adjusting the period of the mean shape to each pulse and scaling the pulse shape by a linear regression. The log of the AC and DC values was used.

The data were detrended by a digital high-pass filter with a variable cutoff frequency. The filter was very sharp ($\sim$10 dB/0.1 Hz). As a cutoff frequency we selected 2.2 times the mean heart rate, in order to further reduce the effect of the hemodynamic pulsatility, but still to be low enough not to affect the fast neuronal signal at 2.5 times the mean heart rate. This filter also removes low-frequency effects, such as breathing and slow cerebral vasomotion.

The cross-correlation function (CCF) between the optical data (OD) and the tapping signal (TS) was calculated according to the following equation: $CCF(\tau) = \Sigma[OD(t)*TS(t+\tau)]/\Sigma TS(t)$. The advantage of this equation with this particular normalization is that it removes the amplitude of the tapping signal and preserves the amplitude of the optical data. The CCF effectively extracts signal components in the optical signal, which are coherent with the tapping signal even if the tapping is not perfectly periodical. Compared to a time-triggered average, a CCF yields defined values not only for the stimulation, but also for the rest periods.

It is of great importance to carry out a noise analysis of the optical data. More than half of the light bundles contain pure noise, because they do not interrogate activated tissue. In such a case it is useless to carry out further analysis, e.g., a fast Fourier transform (FFT), because the amplitude and phase calculated by the FFT would be random and completely meaningless. To average such data with data from an activated area would result in errors. It would also be impossible to calculate the signal-to-noise ratio, if there was no significant signal. It is important to demonstrate that a signal in a light bundle is not random, i.e., it is a real signal. To determine which light bundles contain a real signal and which contain noise, we carried out the following noise analysis. The tapping signal was also cross-correlated to optical signals measured on a solid silicon block to obtain a reference

measurement of pure noise. The level of this noise was determined before and after taking the CCF depending on the number of samples. In our case, the CCF obeys the statistical laws, i.e., the factor by which the level of random noise is reduced depends on $1/N^{1/2}$, where N is the number of data points. In other words, because of the random nature of noise, it will cancel out more the more data are averaged, i.e., the more data points are included in the CCF. In contrast, if the optical data contain components which are coherent with the tapping signal, this genuine signal will keep its amplitude independently of the number of data points. The measurements on the solid silicon block were used to determine the factor of the noise reduction depending on N. It was verified that this factor also holds for physiological measurements. Thus it was possible to identify light bundles, which contained signals, which were statistically significant above this level of noise.

To investigate the reproducibility of such signals, we split data sets in thirds and halves in order to determine their change in signal amplitude and in signal-to-noise ratio.

To better determine the relation between the tapping and the detected signal, we compared the signal amplitudes during tapping and rest.

To analyze the location of the signals we generated back-projections (pixel size, 8 mm) of the signals. For this purpose, we took all significant ($P < 0.001$) DC light bundles, which had at least a factor 1.5 higher amplitudes during tapping than during rest. We took an FFT of each bundle for -2.5 s $< \tau < 2.5$ s, which yielded us the amplitude of the signal at the tapping frequency and the phase of the signal with respect to the tap, which corresponds to the latency of the signal.

Slow hemodynamic signal. After filtering out the heart pulse as described above, the sample rate of the data was reduced to 9.6 Hz by averaging 10 samples and the natural logarithm was taken. A folding average was taken to convert the whole stimulation sequence into one corresponding to the tapping and a rest period. The time traces of these signals were visually inspected and corresponded to well-known patterns of increase in O_2Hb and decrease in HHb (Maki *et al.,* 1995; Hirth *et al.,* 1996; Obrig *et al.,* 1996; Toronov *et al.,* 2000, 2001). The last 10 s of the tapping and the last 10 s of the rest period just before the beginning of the tapping were averaged. The average of the rest period was subtracted from the average of the tapping period. The resulting difference, which corresponds to the change in absorption due to the stimulation, was backprojected (pixel size, 8 mm, as above) separately for each wavelength (758 and 830 nm). For each pixel the changes in O_2Hb (in μmol/liter) and HHb (in μmol/liter) concentrations were calculated from the absorption backprojections using the differential path length factor method (Delpy, 1988).

RESULTS

Significance of Light Bundles

A total of 25 stimulation runs were carried out in the five subjects. Theoretically, in our frequency-domain instrument we could simultaneously measure the AC, DC, and ϕ of one bundle per source detector combination, which would lead to

FAST NEURONAL SIGNALS DETECTED BY NIRS
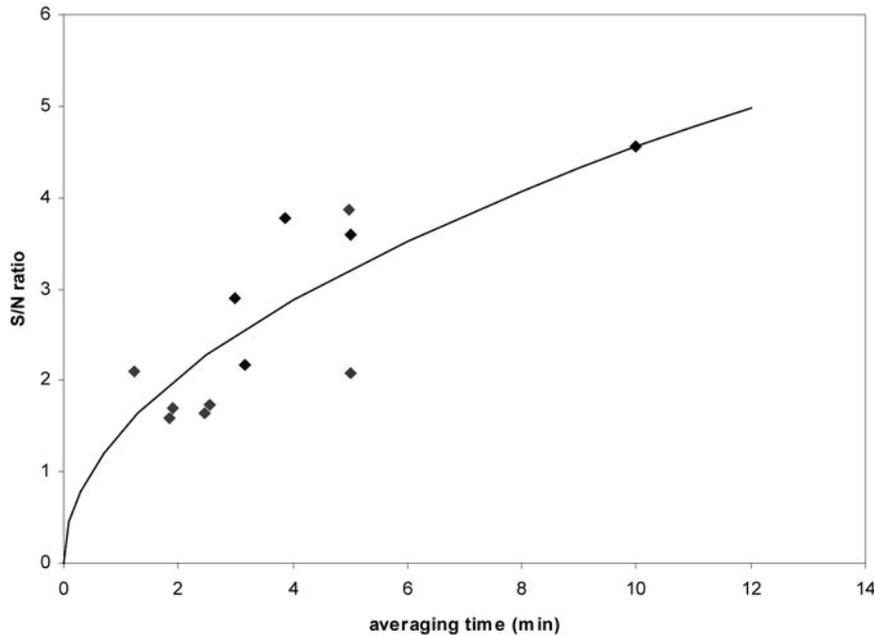
FIG. 2. The mean signal-to-noise ratio with respect to the averaging time increases as expected. This graph was generated from the data of two subjects. The signal-to-noise ratio was averaged over all light bundles with a significant signal. The data set was split into two and three parts to calculate the signal-to-noise ratio of shorter periods of time. The signal-to-noise ratio increases with the square root (indicated by the line) of the measurement time, which is as expected for a real signal. Furthermore, from this graph we determined, that 5 min of measurement per condition was appropriate for our study.

a total of 16 light bundles. In practice, in some measurements the signal at the two shortest source detector distances was out of the dynamic range of the A/D card. Thus we had a total 367 AC, DC, and ϕ signals for the 25 runs.

The instrumental noise of the control data (averaged over a time equivalent to the stimulation period) was very low (mean AC, $0.0021 \pm 0.0013\%$ SD; DC, $0.00052 \pm 0.00032\%$; and ϕ, $0.0012 \pm 0.00059°$).

We found 25 AC, 134 DC, and 1 ϕ (always out of 367) which were significantly different from noise at a level of $P < 0.001$ and 54 AC, 174 DC, and 10 ϕ which were significant at a level of $P < 0.05$. We found significant AC bundles in 14 ($P < 0.001$) or 21 ($P < 0.05$) of 25 stimulation runs. Significant DC bundles were found in every run. For $P < 0.001$ the mean amplitudes $\pm$ standard deviation were AC, $0.0093 \pm 0.0053\%$; DC, $0.0056 \pm 0.0034\%$; and ϕ, $0.0046°$. For $P < 0.05$ the values were AC, $0.012 \pm 0.012\%$; DC, $0.0060 \pm 0.0046\%$; and ϕ, $0.0078 \pm 0.0052°$.

Signal-to-Noise Ratio

When the number of data points was reduced by a factor of 2 or 3, we found less than 20% of variation in the signal amplitude of significant signals. More importantly, the signal-to-noise ratio increased with the number of data points as expected (Fig. 2).

Tapping and Rest Periods

Each stimulation period consisted of blocks of 20 s of tapping and 20 s of rest and the amplitude of the signal during tapping was compared to the one during rest. In terms of the

CCF this concerns its amplitude for -1 s $< \tau < 1$ s (tapping) and -21 s $< \tau < -19$ s or 19 s $< \tau < 21$ s (rest). We found that for a significance level of $P < 0.001$ in 20 of the 25 AC bundles, 90 of the 134 DC, and 1 of 1 ϕ bundles the amplitude during tapping was at least 1.5 times larger than that at rest (for $P < 0.05$, AC, 40 of 54; DC, 107 of 174; ϕ, 6 of 10). For $P < 0.001$ the AC, DC, and ϕ amplitudes were 2.63, 2.67, and 1.95, mean times higher during tapping than during rest, respectively.

Latencies

In general we found good agreement among latencies for a given run. The mean within standard deviation was 16 ms. The mean time when the maximum of the CCF appeared was -25 ± 82 ms before the fingers met. The large standard deviation is due to interindividual differences. The minimum in the CCF occurred at 139 ± 85 ms (Fig. 3).

Location

The localization of the fast and slow signals is displayed in Fig. 4 and 5; 81.6% of the significant fast signals were colocalized with the slow signal. This percentage increased if we included only higher amplitudes of fast signals. In contrast 52.8% of the slow signals were not colocalized with a fast signal.

Slow Signal

The backprojections of the HHb concentration changes during stimulation are shown in Figs. 4 and 5. The changes
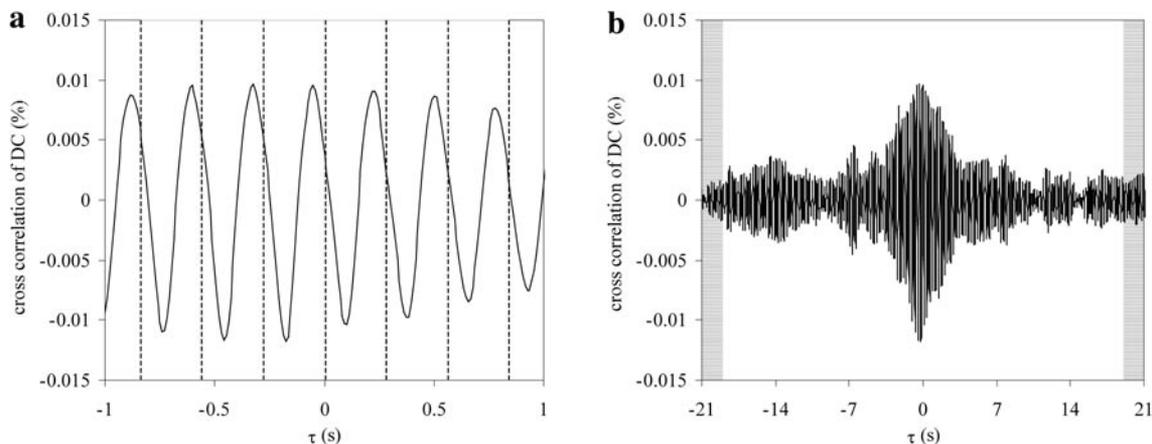
FIG. 3. A cross-correlation function (CCF) showing a fast neuronal signal. The same is depicted at higher (a) and lower (b) resolutions. The CCF in (a) corresponds to a smoothed averaged response of the optical signal to the tapping; τ denotes the period by which the tapping signal was shifted with respect to the optical signal and is equivalent to the measurement time in this case. As expected the signal is particularly strong for a τ between -1 and $+1$ s, when the tapping and the optical signal are synchronous. At $\tau = \pm20$ s the amplitude during rest can be seen. This amplitude is much smaller than that of the fast neuronal signal. Broken vertical lines indicate the moment when the fingers meet (a). Shaded areas (b) indicate the rest periods.

in O_2Hb looked exactly the opposite. Although the localization of the two patterns was very similar, there were differences in the time course, as observed previously (Maki *et al.,* 1995; Hirth *et al.,* 1996; Colier *et al.,* 1999; Obrig *et al.,* 1996; Franceschini *et al.,* 2000; Toronov *et al.,* 2000, 2001): the O_2Hb reached its maximum clearly before the HHb reached its minimum.

DISCUSSION

Our system was highly noise optimized using an increased light intensity to reduce shot noise, with highly sensitive photomultiplier tubes as detectors, a tapping frequency that minimized physiological noise, special filtering, and data analysis based on cross-correlation algorithms. Thus we were able to achieve very low noise levels as tested on a solid silicon block, which were much lower than the fast signals detected.

To ensure that our signals were not due to noise, we studied the behavior of the CCF. As expected the factor by which the level of noise is reduced by the CCF depends on the number of data points. In contrast, a genuine signal which is coherent with the tapping will keep its amplitude independently of the number of data points, provided its amplitude is higher than the noise level. This difference in the behavior of a real signal and noise enabled us to distinguish them and to attribute a statistical significance to the amplitude of a signal. As reported, we found many highly significant signals which were clearly not noise.

How do these signals relate to the finger tapping? To answer this question, the finger tapping was conducted in a rhythm of 20 s of tapping and 20 s of rest. A signal associated with finger tapping will show a higher amplitude during the tapping than during the rest period. The majority of the significant signals showed this behavior. However, some signals had a similar amplitude during the tapping and rest period. As stated above these signals were clearly not random. They indicate that there is background activity which is at least partially coherent with or has similar frequency components to the tapping function. It is beyond the scope of this paper to precisely identify the origin of this background signal, which will be an interesting subject for further studies. The background signals are limited to a few light bundles, i.e., they are localized and not systemic. This excludes the breathing or heart pulse as an origin. The signals are a local phenomenon which becomes clearly detectable due to our highly sensitive system.

A look at the backprojections reveals that the fast signals, which have higher amplitude during tapping than rest, are highly localized. They are much more localized than the slow signal even though they originate in a similar area. As expected (Malonek and Grinvald, 1996), the slow signal affects a larger area. Thus the neuronal activity (origin of fast signal) concerns a small volume of tissue, but increases hemodynamics in a much larger area (origin of slow signal) to provide oxygen and metabolic supply for the region of neural activity. From this point of view, the regions of the fast and slow signals are reasonably related.

If the fast signal detected were a movement artifact by direct mechanical coupling between the sensor and the moving finger, it would not be localized. Furthermore, the spatial pattern depends on the type of stimulation. Within one run, the amplitudes of the bundles were constant. Thus we can exclude a movement artifact. The reasonable spatial agreement between the fast and the slow signals argues for a neural origin of the fast signal. Furthermore, the fast signals of a particular run exhibit similar latencies, which is another indication that they are not random.

In summary, the fast signal described cannot be due to random noise because it has a significant amplitude, similar latencies for the same run, and disappears during rest periods. It cannot be caused by movement artifacts because it shows a distinct spatial pattern which depends on the type of stimulation. The localization of the fast signal is in reason-
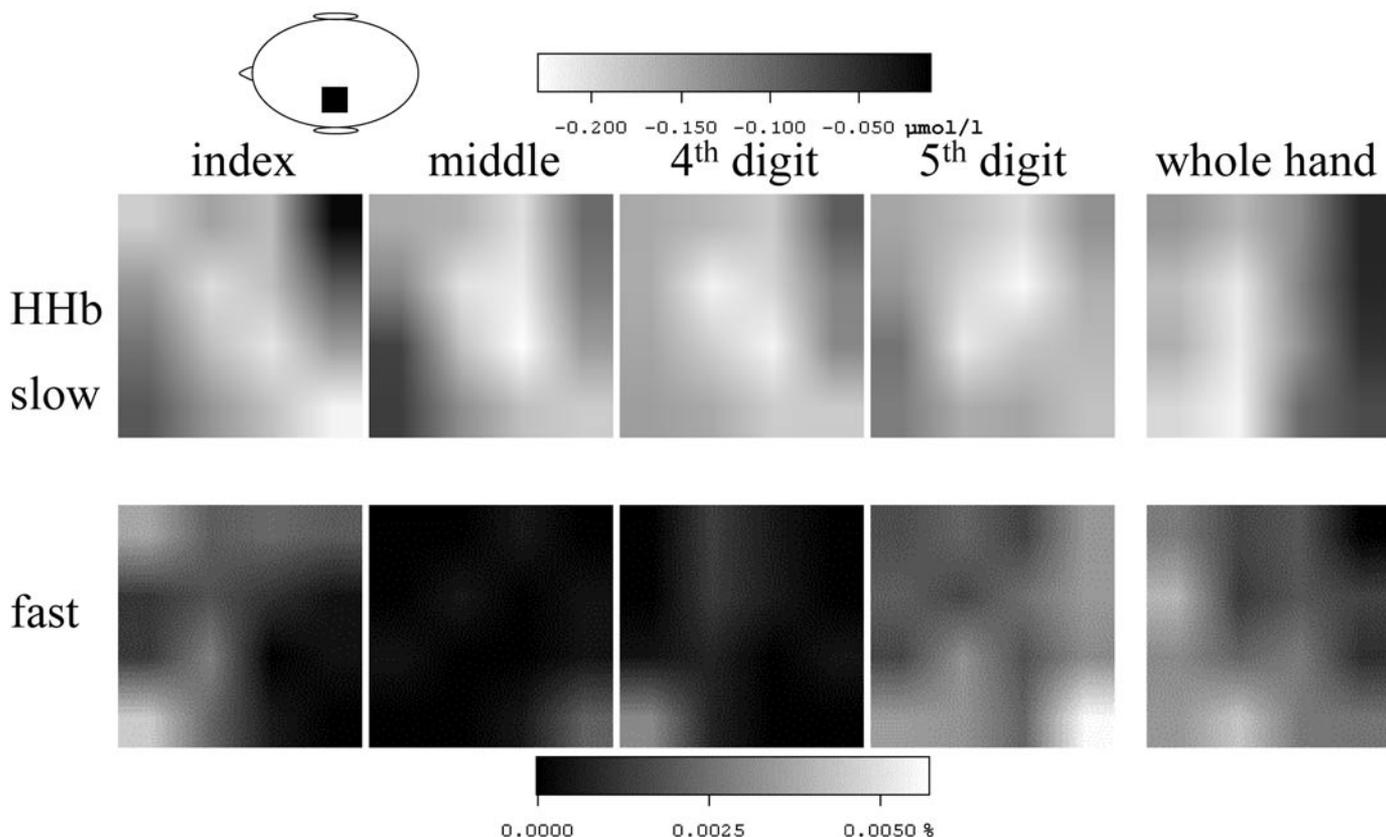
FIG. 4. Maps of the slow hemodynamic and the fast neuronal signals for a male subject of 36 years. The diagram on top depicts the head of the subject with the approximate area of the measurement, which is represented by the maps. In general a white spot indicates an area of strong activation, while a dark area represents no activation. The top row shows the maps for the changes in HHb concentration (slow signal) and the bottom row the fast signal, each with the corresponding scale. Each column represents a different stimulation with one of the indicated fingers or the whole hand. The measurement with the whole hand was carried out on a different day and the probe may not have been at exactly same location (within 15 mm) as that for the other fingers. All stimulations with individual fingers were recorded without moving the probe. The slow signal is not very localized, i.e., the patterns for the different fingers look similar and a broad area lights up. The fast signal is very localized, i.e., a much smaller area lights up and clearly shows individual spots of activity depending on the finger.

able agreement with the slow signal. For all these reasons we conclude that the fast signal originates from neuronal activity.

What is the physiological origin of these fast changes in the optical properties of neuronal tissue?

There have been several studies on the origin of the fast signal in single nerve cells in animals and humans. Extensive reviews can be found in Cohen (1973), Hochman (1997), and Tasaki (1999). The main point of view in this discussion is the origin of the optical signal and what kind of optical signal we should expect: Is it a change in absorption or scattering and do we expect an increase or decrease?

Cohen (1973) found voltage-dependent changes in birefringence in the membrane of nerve cells. He suggested that the observed effect was due to either a thinning of the membrane or a Kerr effect, i.e., that molecules in the membrane are aligned by the electric field. Stepnoski *et al.* (1991) also observed a change in birefringence located in the membrane of a nerve cell, but did not offer any explanation for it. The changes in birefringence were practically simultaneous with the action potential. From this literature we derive that an action potential leads to a decrease in birefringence, which

leads to an increase in light scattering (Stepnoski *et al.,* 1991). Tasaki (1999) found that thermal (heat production and absorption), mechanical (swelling and shrinking), and optical (birefringence) changes in the axon were simultaneous and related to a sudden swelling of the gel layer of the membrane due to an increase in water content. This gel layer was superficial, about 0.5 μm thick, and an integral part of the membrane of the axon.

Cohen (1973) also reported a biphasic change in light scattering (90°), i.e., an increase in light scattering, which was simultaneous with the action potential; after a return to the baseline another increase in light scattering was observed with a peak at 20 ms after the action potential. He suggested that these changes are current and volume dependent.

It is difficult to extrapolate the data cited on single nerve cell preparations to our situation because a change in birefringence cannot be related in a straightforward manner to a change in light scattering. Cohen's delayed increase in light scattering (90°) has not been confirmed by newer literature. None of the authors mentioned any changes in light absorption, which does not necessarily mean that there is no change in light absorption. Salzberg *et al.* (1985) studied slices of the
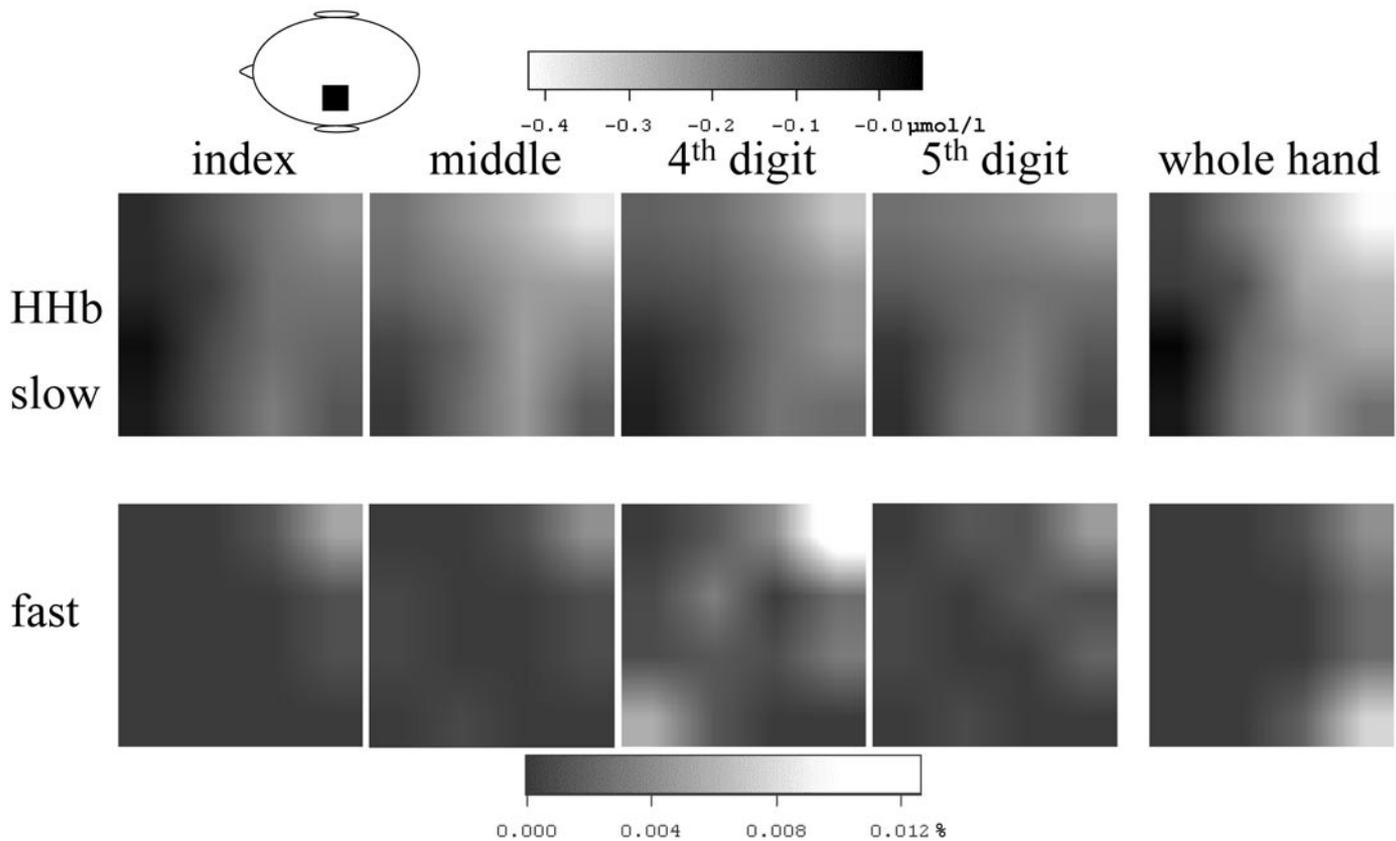
FIG. 5. Maps of the slow hemodynamic and the fast neuronal signal for a female subject of 30 years. The figure is analogous to Fig. 4 except that all data were recorded without moving the probe. Again the fast signal exhibits a much more localized pattern than the slow signal, i.e., a smaller area lights up. In this case the fast signal shows less variation depending on the finger compared to Fig. 4 and the right upper corner always lights up. This indicates that we mostly see the activations from the thumb, which was tapping against the other fingers.

neurohypophysis of mice and reported large and rapid increases in light scattering, which accompanied the secretion by nerve terminals. These changes occurred in two phases and the fractional light intensity change was approximately 0.2%.

Further work has been done in animals, as on the open cat cortex by Grinvald *et al.* (1986), Rector *et al.* (1997), and Malonek and Grinvald (1997). Grinvald *et al.* (1986) reported a decrease in reflected light 200 ms after the onset of whisker stimulation. Rector *et al.* (1997) found a decrease in reflected light synchronous with the electrical signal. These findings can be interpreted as either a decrease in light scattering or an increase in light absorption.

Malonek and Grinvald (1997) reported an increase in light scattering 200 ms after the onset of stimulation.

In humans Gratton *et al.* (1995, 1997a,b,c, 2000; Gratton and Fabiani, 2001), Rinne *et al.* (1999), and DeSoto *et al.* (2001) report increases in the phase of a frequency-domain NIRS instrument. This can be interpreted as either an increase in light scattering or a decrease in light absorption. Steinbrink *et al.* (2000) reported a decrease in reflected light, which suggests an increase in light scattering or absorption.

Although all authors of the literature cited assumed a change in light scattering from the methodological point of view it cannot be excluded that the reported results (except those of Salzberg *et al.* (1985) and Stepnoski *et al.* (1991), who

looks only at isolated cell membranes) are also subject to a change in light absorption. Furthermore, it is not clear whether the scattering increases or decreases.

In our data we find that the CCFs of the significant AC or DC bundles at 758 nm have similar latencies and amplitudes and are in phase with the ones at 830 nm. The CCF of the significant ϕ bundles and their corresponding DC bundles are nearly in phase. These observations could indicate that the change in the optical properties is not due to light scattering, but to absorption, in a time scale different for the hemodynamics changes.

The changes in the optical properties that we have observed have a lower amplitude than the ones reported for DC (Steinbrink *et al.,* 2000) or ϕ (Gratton *et al.,* 1995, 1997a,b,c, 2000; Gratton and Fabiani, 2001; Rinne *et al.,* 1999; DeSoto *et al.,* 2001). The main reason is that our system has very low noise, which enabled us to detect lower signal amplitudes. Steinbrink *et al.* (2000) used a much stronger stimulus (electrical stimulation), which presumably will lead to a higher signal amplitude.

CONCLUSION

We have been able to considerably reduce the noise in the NIR frequency-domain instrument system and improve data