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.

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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$_2$Hb) 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
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 fiber optics. 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 (SaO₂) 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

**FIG. 1.** Our frequency-domain near-infrared spectrophotometer (Oxy-Imager, ISS Champaign, IL) with a diagram of the sensor is shown.
signals measured on a solid silicon block to obtain a reference which contains noise, we carried out the following noise analysis to determine which light bundles contain a real signal and not random, i.e., it is a real signal. To this end, we normalized the measured signals, which were statistically significant above this level of noise.

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 (~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(τ) = \sum [OD(t) \cdot TS(t-τ)] / \sum OD(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 periodic. 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 optical data. More than half of the light bundles contain pure noise, which are coherent with the tapping signal even if the tapping is not perfectly periodic. Compared to a time-triggered average, a CCF yields defined values not only for the stimulation, but also for the rest periods.

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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 /H9278 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 ± 0.0013% SD; DC, 0.00052 ± 0.00032%;
and /H9278, 0.0012 ± 0.00059°).

We found 25 AC, 134 DC, and 1 /H9278 (always out of 367) which
were signiﬁcantly different from noise at a level of
P = 0.001 and 54 AC, 174 DC, and 10 /H9278 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 ± standard deviation were AC, 0.0093 ± 0.0053%; DC, 0.0056 ± 0.0034%; and /H9278, 0.0046°. For P < 0.05
the values were AC, 0.012 ± 0.012%; DC, 0.0060 ± 0.0046%;
and /H9278, 0.0078 ± 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 sig-
nal-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 tap-
ing 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 < τ < 1 s (tapping)
and −21 s < τ < −19 s or 19 s < τ < 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 /H9278 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; /H9278, 6 of 10). For P <
0.001 the AC, DC, and /H9278 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 colo-
calized 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
in O$_2$Hb 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$_2$Hb 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-
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 signals we should expect: is it a change in absorption or scattering and do we expect an increase or decrease?

Cohen (1973) and Hochman (1997) found voltage-dependent changes in birefringence in the membrane of nerve cells. Cohen 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. Cohen 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. 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 Hb 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.](image-url)
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 $\phi$ 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 $\phi$ (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...
analysis algorithms. With the signal-to-noise ratio achieved, single subject measurements become feasible without the requirement of particularly strong stimuli and within a reasonable period of measurement of 5 min. The reported fast signals are significantly higher than noise and exhibit much more localized patterns than the slow hemodynamic signals. The great advantage of this noninvasive technique is that it is able to directly detect neuronal activity with a relatively high spatial resolution.

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