FAST OPTICAL SIGNAL DETECTED IN THE PREFRONTAL LOBE WITH NEAR-INFRARED SPECTROSCOPY DURING SLEEP

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By

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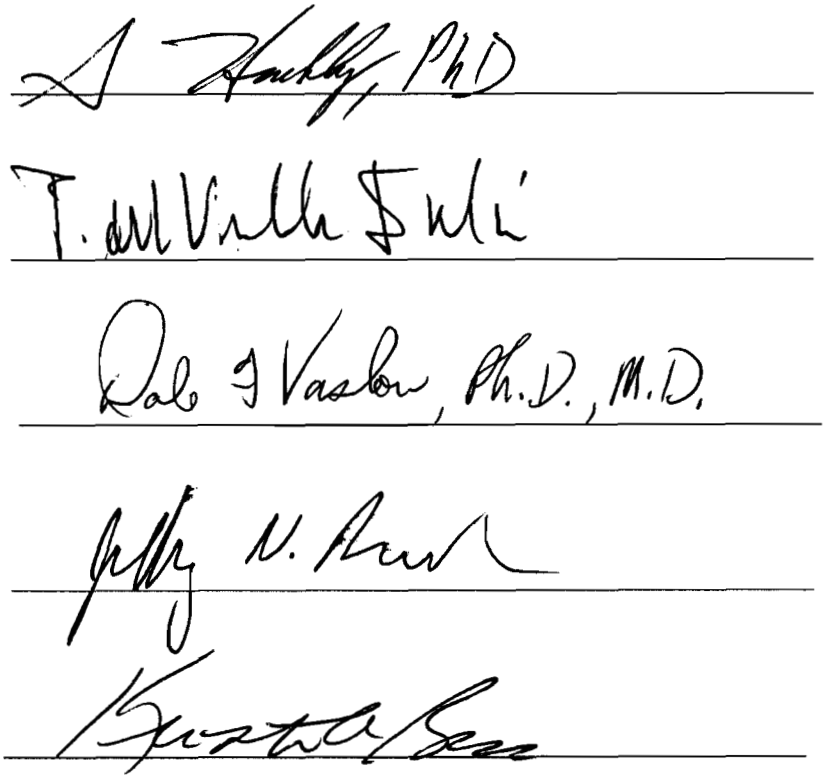
Presented by Jun Zhao

A Candidate for the degree of Master of Arts

And hereby certify that in their opinion it is worthy of acceptance.



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I dedicate this thesis to the memory of my father, Zhao Jia Tao.

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WITH NEAR-INFRARED SPECTROSCOPY DURING SLEEP

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ABSTRACT

If near-infrared spectroscopy (NIRS) is to be used in clinical applications such as the localization of epileptic foci, it must be capable of recording large amplitude transients of which only a few samples are available. With this in mind, we attempted to record the NIRS correlate of isolated delta waves during normal human sleep. Large-amplitude, isolated delta waves in the electroencephalogram (EEG) were selected and the corresponding optical responses were measured. Signal-averaging trials of delta waves revealed fast optical intensity changes ranging from 0.05% to 0.3% but of unstable morphology. Measuring from the positive peak of the delta wave to the nadir of the individual optical responses, we were able to detect a latency of approximately 130 to 180 ms in 75% of the channels. Although encouraging, the results implied that the signal-to-noise ratio of NIRS is not yet adequate for clinical application.

Introduction

There are several functional brain imaging methods used to investigate brain activity. The most common imaging techniques can be classified in terms of whether they measure hemodynamic or neuronal activation. Hemodynamic methods, such as positron emission tomography measure cerebral metabolic rate of oxygen and cerebral blood flow, and functional magnetic resonance imaging (fMRI), which measures blood flow and blood oxygenation level dependent (BOLD) changes, have excellent spatial resolution but limited temporal specificity. In contrast to hemodynamic methods, magnetoencephalography (MEG) and electroencephalography (EEG), which measure neuronal activation directly through electrical and magnetic fields, have excellent temporal resolution, but poor spatial resolution. By using these techniques, great advances have been made in the investigation of the human brain. However, it would be advantageous to be able to image both neuronal and hemodynamic activity simultaneously.

Near-infrared spectroscopy (NIRS) has shown potential to do just that. Biological tissue is relatively transparent in the near-infrared range, between 700 and 1000 nm (Villringer & Chance, 1997), due to the relatively low absorption of in this wavelength range (Wray et al., 1988). Consequently, near-infrared light can penetrate biological tissues to an appreciable depths, thereby opening an "optical window" to assess brain tissue noninvasively (Jobsis, 1977).

It has been well documented that NIRS is a useful technique for investigating cerebral hemodynamic changes in the human brain (Colier et al., 1999; Hoshi & Tamura,

1993; Villringer et al., 1993). The hemodynamic changes measured with NIRS are often called *slow signals*, because they occur within seconds after the brain activity begins. Some researchers have suggested that slow signals correlate with the BOLD fMRI signal (Strangman et al., 2002; Toronov et al., 2001) and result mainly from changes in the concentration of endogenous absorbers, such as oxyhemoglobin and deoxyhemoglobin (Gratton & Fabiani, 2003; Jobsis, 1977; Villringer, 1997).

A more controversial question is whether NIRS can measure neuronal activity directly. It was shown as early as 1949 that the action potentials of nerve cells are associated with changes in light scattering (Cohen & Keynes, 1971; Hill & Keynes, 1949; see also Stepnoski et al., 1991). Similarly, decreased reflectance was found in guinea pig cerebral cortical slices when cell membranes were depolarized (Lipton, 1973). Invasive methods have shown fluctuations in the optical properties of brain cells within in vitro preparations, including brain slices, and in intact cortical tissue (Cohen, 1973; Grinvald et al., 1986; Harik et al., 1979; Jobsis, 1977; Salzberg & Obaid, 1988). Recordings from exposed cortical tissue in animals have associated fast light reflectance with electroencephalographic patterns (Rector, et al., 1995). Finally, noninvasive measurements in human subjects have also shown that NIRS has the potential to noinvasively measure neuronal activity (Franceschini & Boas, 2004; Gratton et al., 1995; Gratton et al., 2001; Rector et al., 1995; Steinbrink et al., 2000).

Compared with the slow hemodynamic signal, the *fast signal* in NIRS occurs within milliseconds after the stimulation. Research suggests that this signal originates from action potentials and the consequent swelling of the neuronal cells or from a brief period of anaerobic metabolism that somehow alters tissue transparency (Cannestra et al.,

2001; Cohen, 1973; Malonek & Grinvald, 1996; Rector et al., 1995). By using a frequency-domain instrument, which employs light sources that are modulated at more than 100 MHz, and a π-sensor, which is composed of two crossed source-detector pairs, Wolf et al. (2003) reported a fast optical signal in the 100-ms range during visual stimulation generated by a checkerboard. With a similar but uncrossed system, Gratton and colleagues measured a fast change in the phase of detected relative to incident light within the motor, visual, and auditory cortices (Gratton et al., 1995; Rinne et al., 1999). Both Steinbrink et al. (2000) and Franceschini and Boas (2004) detected fast optical intensity changes following electrical median nerve stimulation using a continuous-wave recording system (i.e., either a steady source of light or light modulated at a frequency less than 100 MHz).

Because the expected fast optical signals are usually much smaller (i.e., by two orders of magnitude) than the noise level, various strategies must be used to maximize the signal-to-noise ratio (SNR). Whether the solution is applying a more powerful emitting source and photomultiplier tube (PMT) detectors (Steinbrink et al., 2000), using multiple source-detector pairs (Franceschini & Boas, 2004), or special π-sensors (Wolf et al., 2003), the low SNR of noninvasive optical measurment of the fast signal remains a challenging problem.

Another approach to this problem is to average across large numbers of trials, as is commonly done in fMRI or event-related potential research. Event-related potentials occur as small fluctuations in EEG recordings associated with sensory, motor, or other mental events (Handy, 2004). Event-related potentials (ERPs) are quite small in the amplitude (1 to 5 μV) compared to background EEG signal (10 to 100 μV; Gratton &

Fabiani, 2003). On an EEG recording, these event-related fluctuations are difficult to detect, because they are often masked by electrical signals generated by other, unrelated brain activity. To "unmask" the true ERP, it is necessary to take recordings from repeated presentations of the stimulus in order for a computer to produce an averaged ERP waveform. Anywhere from a few dozen trial (for cortical components) to several hundred (for brainstem potentials) are required. Similarly, because optical signals are submerged into the huge arterial pulsation noise, hundreds of trials are required to show the fast signal. Because the main challenge to measuring noninvasive optical signals in humans is low SNR (signal-to-noise ratio), we used a frequency-domain instrument to measure the fast optical intensity signal that correlates with the huge, spontaneous delta waves in the sleep EEG.

Grey Walter (1936) coined the term *delta waves* to refer to particular types of large slow waves recorded in the EEG of humans. They occur mostly in Stage 3 and Stage 4 sleep, which are together referred to as slow wave sleep (SWS). The classical frequency band is from 0.5 to 4 Hz, with amplitudes ranging from 75 to 200 μV (Amzica & Steriade, 1998). The genesis of a delta wave involves both cortically-generated and thalamic oscillations. With the greater amplitudes of delta waves, which are 10 to 100 times larger than those of ERPs, we should be able to greatly increase the SNR of the optical signal and thereby assess its utility for detecting large amplitude pathological transients, such as epileptic discharges.

NIRS has been used before to investigate animal and human sleep. In 1991, Onoe (1991) studied rapid eye movement (REM) sleep–associated hemoglobin oxygenation changes in the forebrain of monkeys. In humans, Hoshi et al. (1994) and Spielman et al.

(2000) found hemodynamic changes during sleep and during the transition between wakefulness and sleep using near-infrared spectroscopy. By studying the exposed cortical tissue of sleeping cats, optical reflectance changes were revealed within the slow wave band using spectral estimates (Rector et al., 1995). Because the optical reflectance change in that study were observed in active (i.e., REM) sleep as well as in quiet (i.e., nREM) sleep, the relation of these fast signals to human delta waves is unclear.

In our study, we measured the fast signal during SWS and specifically related to the findings to electrically recorded delta waves. Because hair can absorb much of the light being emitted from the near-infrared sources (Gratton et al., 1997), we measured the signal from the forehead. The frontal lobes were also selected because of their predominance in generating EEG delta activity during sleep (Cajochen et al., 1999; Munch et al., 2004).

Methods

Participants

The final sample consisted of four healthy adult volunteers (two men and two women, ages 18 to 23 years), who were recruited from an introductory psychology class. They received extra credit points in this class in recognition of the educational value of research participation. All subjects were asked to restrict their sleep time to a maximum of 6 hours the night before the experiment to facilitate falling asleep. All of the subjects gave written informed consent prior to participation. There were 26 additional subjects whose data were not analyzed. Among these, 24 were unable to fall asleep because of the discomfort produced by the optical sensors, and 2 were rejected due to equipment problems.

Apparatus

Optical Recording

The optical data were recorded with a 16-channel OMNIA Tissue Oxymeter (ISS Inc., Champaign, IL) using the frequency-domain method. The sources were laser diodes that emit light at 750 nm (near-infrared range), with a power of ~1 mW (less than that of a penlight). The intensity of light was modulated at 110 MHz and channeled through optical fibers, 0.4 mm in diameter. Four optic fibers were held in place by metal retainers (4 to 10 mm diameter) mounted on a 7 x 10 x 0.5 cm convex, rectangular piece of plastic. The distance between optical fibers to detectors ranged from 3 to 3.5 cm. Two optical source fibers were positioned above the detector (i.e., higher on the forehead) and the other two were below. The average recording depth of brain activity was estimated at half this distance (i.e., about 1.7 cm; Gratton and Fabiani, 1998). The padded plastic mount

was held on the forehead by an elastic band, with the detector centered about 5 cm above the nasion.

The detectors were two optical bundles with a diameter of 3 mm connected to a photomultiplier tubes (PMT). One of the two detectors was placed on the forehead; the other recorded a red light emitting diode (LED) signal that pulsed every 5 seconds as a time marker. The LED pulse was simultaneously recorded electrically using a photovoltaic cell in order to synchronize the EEG and optical signals. A portion of the optical signal was sent to the PMT of the oxymeter and was subtracted from a 110.005 MHz constant source to generate a difference signal, similar to the beat frequency produced by a tuning fork and an out-of-tune piano string. This beat, or *heterodyning*, frequency (5 kHz), is low enough to be digitized for recording. These signals were subjected to fast Fourier transform (FFT) for calculating the relative phase shift, time-averaged intensity (DC) and modulated amplitude (AC). Only DC intensity data are presented here. Because the heartbeat is less regularly represented in an AC signal, it is more difficult to apply the adaptive filter to this analysis. Preliminary analyses indicated that phase had an unacceptably low SNR.

Electrical Recording

A 16-channel EEG amplifier system (Grass Inst. Co.) recorded brain activity using Ag/AgCl disk electrodes attached to the scalp. The following montage was used, according to the international 10-20 system: F7, F8, C3, Oz, Fz, Fpz' (in the middle of the optical array on the forehead), left outer canthus, right outer canthus, superior orbit, and inferior orbit, all of which were referenced to the left mastoid during acquisition. The four periocular sites were re-referenced into bipolar derivations for vertical and

horizontal electro-oculograms (vEOG/hEOG) to record the eye position. A bipolar derivation recorded chin muscle activity electromyographically. All impedances were kept below 10 kOhm. The scalp electrodes, electro-oculograms and electromyogram were used for identifying the stage of sleep. Because of its superior SNR, the C3 was used for recording delta waves to compare with the optical signal.

Protocol

Subjects were told to reduce their sleep time to a maximum of 6 hours the night before the experiment. The protocol began at their normal bed time. After application of the sensors, the participant lay on a comfortable reclining chair in a quiet dark room. The recordings began before the participant fell asleep. When the polygraphic record indicated that the participant had finished the first round of stage 3 and 4 sleep, recording continued for another 30 minutes, at which time he or she was awakened. The stage of sleep was assessed according to the criteria of Rechtshaffen and Kales (1968), except that a bipolar derivation was used for the electrooculograms.

Data Analysis

The optical raw data were sampled at 25 Hz. A high-pass filter (cutoff 0.5 Hz) was used to eliminate slow drifts (EEGLAB; Delorme & Makeig, San Diego). To remove the pulse artifact and its harmonics, we employed an adaptive filter (Franceschini & Boas, 2004; Gratton & Corballis, 1995). This filter first identifies each pulse within the DC signal. By warping each pulse interval to a common fixed length, the average pulse is then calculated. Finally, this average then is fitted to each suspected pulse artifact and is subtracted from the raw data (Maclin, et al., 2004).

Concurrent electrophysiological activity was digitized at 100 Hz and band-pass filtered (EMG, 10 Hz to 1 kHz; EEG, 0.1 to 100 Hz; EOG, 0.1 to 100 Hz). In order to temporally synchronize electrical and optical data, we resampled the data offline at 25 Hz, the digitization frequency of the optical signals. Then we used the 5-s LED time marker to precisely align the EEG and optical data.

By applying the signal-averaging method, waveforms of both the averaged EEG and the optical data were obtained. The poor SNR ratio and variable morphology of the resulting waveforms precluded accurate assessment of any time delay between electrical and optical components. Consequently, we conducted a single-trial analysis to compare peak latencies for isolated delta waves. Delta waves usually occur in bursts. If waves were too close to each other, the corresponding optical signal might not reach a baseline between the two contiguous waves. Consequently, we selected delta waves separated by at least 1 s from the previous one. Based on preliminary analyses of individual waves and the signal averaged data, we measured the time point of the lowest signal optical intensity that occurred during each epoch with respect to the positive peak of the delta wave. This positive peak defined the center of the 2-s epoch. Our use of low intensity peak is similar to other fast signals studies that defined latency as the time period from the stimulation to this component of the optical data. Positive delta waves were chosen because surface-positive complexes are believed to represent the depolarization of large pools of layered cortical neurons (Amzica & Steriade, 1998). Paired Student *t* tests were applied to these latency data, separately for each source-detector pair for which a reliable pulse artifact could be observed.

There were four channels could not show reliable pulse artifacts because of poor contacting with the forehead (two for subject A, two for subject B), and were discarded. The other of twelve channels showed reliable pulse artifacts were therefore accepted as good channels.

Results

Figure 1 demonstrates the power spectrum of the unfiltered data (thick line) and of the filtered data (thin line) for one representative participant, Subject C. For the unfiltered data, there are clear peaks at about 1 Hz with strong second, third, and fourth harmonic frequencies, which correlate to the heartbeat. As shown in Figure 1, the adaptive filter succeeded in reducing, by a factor 10 or more, the arterial pulsation intensity fluctuations and its harmonics.

There are other types of physiological background noise which can cause intensity oscillations, such as Mayer waves (low-frequency arterial pressure fluctuations generated from oscillating sympathetic nervous outflow) and respiration. Mayer waves are approximately 0.1 Hz and respiration is about 0.25 Hz. These low-frequency oscillations were removed by using a high-pass filter of 0.5 Hz.

Figure 2 shows the signal-averaged data for a selected channel from one participant (Subject A). The dotted curve represents the average electrical delta wave, which is peak-centered at zero on the X axis. The solid curve in both the left and right panels is the averaged optical data. The data in the right panel have been spline-smoothed. It is clear that the averaged optical data show a decrease in intensity of about 0.3 % in amplitude within 500 ms after the occurrence of the peak of the delta wave. Peak-to-peak amplitudes of the fast optical signal ranged from 0.05% to 0.3% across the 12 accepted channels. Figure 3 shows the similar results as Figure 2, just from another participant (Subject C).

As noted above, morphological variation across subjects and channels precluded using the signal averaged data to estimate the temporal relationship between optical and

electrical transients. One potential problem might be that signal averaging could have mixed optical responses that had enough time to reach the baseline with those that did not. We tried to address this problem using single-trial, peak-to-peak analysis. The latency from the positive electrical peak to the lowest intensity of the optical signal was measured. A paired *t* test was applied to these intervals for each subject and each channel, comparing the observed latency to an expected value of zero. We assumed that if there were no fast optical signal, the smallest intensity value of the noise would be randomly and evenly distributed across the 2-second epoch. The mean of such a uniform distribution would be its center (i.e., a latency of zero).

The results are shown in Table 1. The fast signal was reliably detected in all subjects, but not all channels. The latency varied from 135 to 189 ms across individual subjects and optode locations. The number of epochs included in these analyses ranged from 54 to 77. As reported in Table 1, low-intensity peaks of the optical signal were significantly delayed relative to the positive peak of the electrical signals in 9 out of 12 good channels. A Kruskal-Wallace nonparametric test confirmed that the 12 mean optical peak latencies were significantly greater than the electrical peak latency ($H [1, 24] = 15.4$, $p<0.001$).

Discussion

The experimental results show that NIRS can detect fast signals associated with spontaneous EEG transients, signals that are presumably of neural rather than hemodynamic origin. The characteristics of our putative fast signal are similar to those described in previous reports (Franceschini & Boas, 2004; Maclin, et al., 2004; Steinbrink, et al., 2000; Wolf, et al., 2003): The relative change in intensity is on the order of 0.07% and the latency of the fast response was delayed about 150 ms from the peak electrical activity.

Previous efforts to measure the fast optical signal noninvasively mostly involved ERPs, which are typically quite small in amplitude (1 to 5 μV). Because the correlated optical signal is so small and is submerged in physiological noise, hundreds of trials have to be averaged in order to reach an acceptable SNR. Thus, determining how to increase the SNR to acquire a weak fast signal is still a challenge for such studies. Delta waves characteristically have a huge amplitude (75 to 200 μV), comparable in size to pathological transients such as epileptic discharges. This unique characteristic permitted a smaller number of trials to be used, thereby offering a different perspective on the nature of the fast signal.

Delta Waves and the Fast Signal

The genesis of delta wave involves both cortically and thalamically generated oscillations (Amzica & Steriade, 1998). There are pure, slow (< 1 Hz), cortically generated oscillations, clock-like thalamically generated oscillations, and single-transient cortical delta waves. All of these may coalesce to produce a pattern of polymorphic delta waves in an EEG recording. The complexity of corticothalamic activity implies that

interpretation of various delta waves should be made with caution. Because the intracortical field potentials are reversed at the surface of the cortex for deep sources (e.g., somatic postsynaptic potentials in pyramidal cells), surface-positive complexes represent the *depolarization* in large pools of layered cortical neurons (Amzica & Steriade). To simplify interpretation, therefore, we focused on positive rather than the negative peak of delta waves in the scalp EEG.

Optical measurements of slow hemodynamic changes have previously been reported in a few animal and human sleep studies (Hoshi et al., 1994; Onoe et al., 1991; Spielman et al. 2000), but fast neuronal changes, only in a single study. By using spectral analyses of the EEG in cats, Rector et al. (1995) showed that light reflectance changes on the dorsal hippocampal surface correspond to a frequency range similar to that of EEG changes in quiet (SWS) sleep. The authors suggested that the optical changes most likely were derived from fast-acting processes, such as protein conformation changes or cellular swelling, rather than from changes in blood perfusion. In the current study, we measured these changes noninvasively in human subjects. Our finding of a brief delay between the electrical and optical peaks may offer a clue as to the nature of the underlying mechanisms.

Mechanism of the Fast Signal

The finding that the fast signal occurred within 200 ms made it less likely to be a vascular response. The maximal rate of potential vascular changes is assumed to be 0.5 Hz (Lindauer, Villringer et al., 1993), and other studies have shown that a significant optical signal remains even in areas without microvasculature or that are subjected to vasodilation blockers (Haglund et al., 1992). Several studies have been conducted to find

the origin of the fast signal in nerve cells, brain slices, and human brains (Cohen & Keynes, 1971; Lipton, 1973). MacVicar and Hochman (1991) reported that light transmission changes that are associated with the cell discharge are abolished if cell swelling is prevented by pharmacologic blockers. According to these authors, a rapid change in refractive properties of the cell membrane is most likely responsible for the fast optical signal (Wolf, et al., 2003).

Franceschini and Boas (2004) reached a different conclusion. They conducted Monte Carlo simulations to test whether the fast signal was the result of changes in light scattering caused by physical changes in the cell membrane or by absorption. If the signal were due to light scattering variations, the simulation indicated that light intensity should show areas of positive and negative changes. But if the signal were due to changes in absorption, the light intensity should show changes in a single direction. Then, in an empirical study with 8 sources and 16 detectors, Franceschini and Boas showed that the fast optical signal increased or decreased at adjacent optodes on the same subject or for the same stimulus in different subjects. Thus, the results were more consistent with focal scattering than absorption changes.

Other physical mechanisms might also explain the origin of the fast signal. Protein conformation alterations might contribute to changes in the light scattering (Landowne, 1992). Because NIRS does not measure the rotation of polarized light, it is not capable of isolating protein conformation changes from other mechanisms. Another possibility concerns synaptic activity. Salzberg et al. (1985) suggested that the secretion at nerve terminals is related to rapid changes in light scattering, producing light intensity changes of about 0.2%.

Detection of the Fast Optical Signal

The fast optical signal we detected is much smaller than slow hemodynamic signals that have been reported in the literature. Because the amplitude of the fast signal is two orders smaller than the physiological noise related to the heartbeat, it was necessary to use a sampling rate higher than 20 Hz and filtered out the pulse and its harmonic artifacts. In previous studies, an average of hundreds of trials was generally necessary to measure the fast optical signal. But these fast optical responses were correlated with ERPs that were quite small in the amplitude (1 to 5 μV). By contrast, we studied delta waves that had amplitudes ranging from 75 to 200 μV. Consequently, we were able to detect the fast optical signal using only 50 to 80 samples per average.

It has been reported that the fast signal shows a latency of about 50 to 180 ms relative to stimulus onset (Franceschini & Boas, 2004; Gratton et al., 1997; Steinbrink et al., 2000;). For example, in a study by Steinbrink et al., a decrease in intensity began 100 ms after stimulus onset and returned to baseline at around 200 ms. In contrast to that study, Franceschini and Boas presented repetitive stimuli at a rate such that the fast optical signal could not reach a baseline from one stimulus to the next. They measured the low intensity peak with a latency of 180 ms for tactile stimulation and tapping and 125 ms for electrical stimulation.

Spontaneous EEG rhythms constitute a similar situation in that the waves normally occur in bursts, so the optical signals might presumably not reach the baseline. Our solution was to measure the latency of the fast signal for isolated delta waves. The results were, nonetheless, similar to those obtained by Franceschini and Boas. The latency of the peak of the optical signal was about 130 to 180 ms.

Our amplitude findings were also similar to those obtained in previous studies of the fast optical signal (Franceschini & Boas, 2004; Steinbrink et al., 2000; Wolf et al., 2003). Although the stimulation in these experiments varied from median nerve stimulation to finger tapping or tactile stimulation, the amplitude changes seemed to be on the same order of magnitude as ours (0.01% to 0.3% vs. 0.05% to 0.3%).

Limitations of the Present Study

The limited size of the array, as well as the fact that it did not include overlapping crossed-pair measurement limited the spatial resolution of the results. Another significant limitation of this study is that the optical system we used only one wavelength (750 nm). Thus, it was not possible to separately estimate the concentration changes of oxy- and deoxyhemoglobin. As a consequence, we could obtain only an overall measurement of tissue transparency changes. With multiple source-detector pairs and more wavelengths, a spatially sensitive map could be created, and oxy- and deoxyhemoglobin could be measured. As noted by Franceschinin and Boas (2004) it may also be feasible to distingush scattering from absorption changes spectroscopically.

Conclusions

In conclusion, although the signal-to-noise ratio appears to be too low to support immediate applications within clinical neurophysiology, improving the ability to simultaneously and noninvasively produce optical measurements of responses to vascular and neural activation will lead to further understanding of the brain's physiologic and psychological processes.

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Table 1.
Mean latency and amplitude of the optical correlate of sleep delta waves for each of the four subjects

Subject	△I/ I 0 (%)	Latency	df	P value
A	0.3	136	53	<.019
A	0.1	180	53	<.004
B	0.06	186	76	<.0001
B	0.02	16	76	N.S.
C	0.09	184	53	<.004
C	0.05	168	53	<.003
C	0.1	135	53	<.02
C	0.05	87	53	N.S.
D	0.06	189	62	<.005
D	0.05	145	62	<.02
D	0.09	179	62	<.005
D	0.05	16	62	N.S.

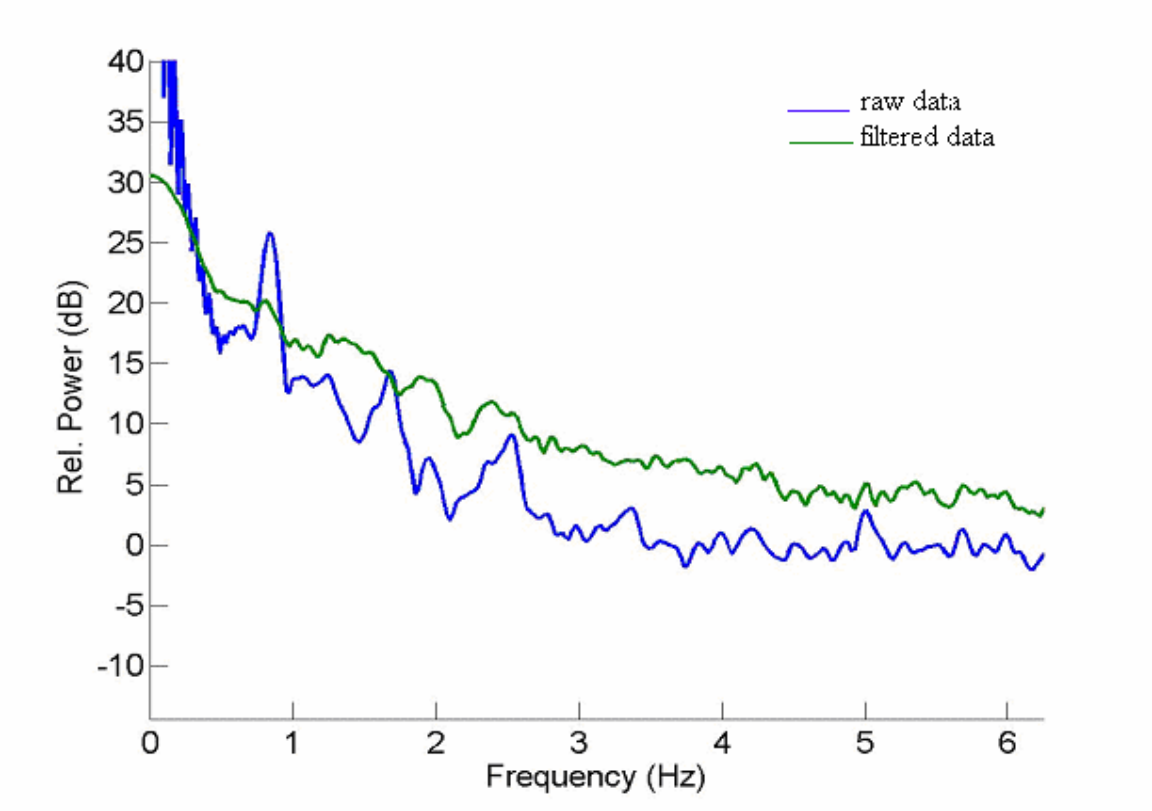
* Note. Latencies are for intensity minima measured with respect to the peak of isolated, surface-positive delta waves; *p*-values are for one-tailed Student *t* tests assessing the null hypothesis that intensity minima occur at random points in time during the 2-s epoch, with a uniform distribution and, consequently, a mean at the center of that epoch.

Figure Captions

Figure 1. Power spectrum of the raw optical data (blue line) and filtered optical data (green line) for a representative participant.

Figure 2. Time traces of the block averaged intensity for delta waves. Fig. 2a(left) shows the results obtained when the optical data are not smoothed. The black curve is the optical data; the dotted curve is electrical delta wave. Delta wave is centered at time zero for the positive peak and has a zero-to-peak amplitude of about 100uV. Fig. 2b (right) shows the same optical data as in Figure 2a, but with smoothing.

Figure 3. Time traces of the block averaged intensity for delta waves. Fig. 3a(left) shows the results obtained when the optical data are not smoothed. The black curve is the optical data; the red curve is electrical delta wave. Fig. 3b (right) shows the same optical data as in Figure 3a, but with smoothing.


raw data
filtered data
Rel. Power (dB)
40
35
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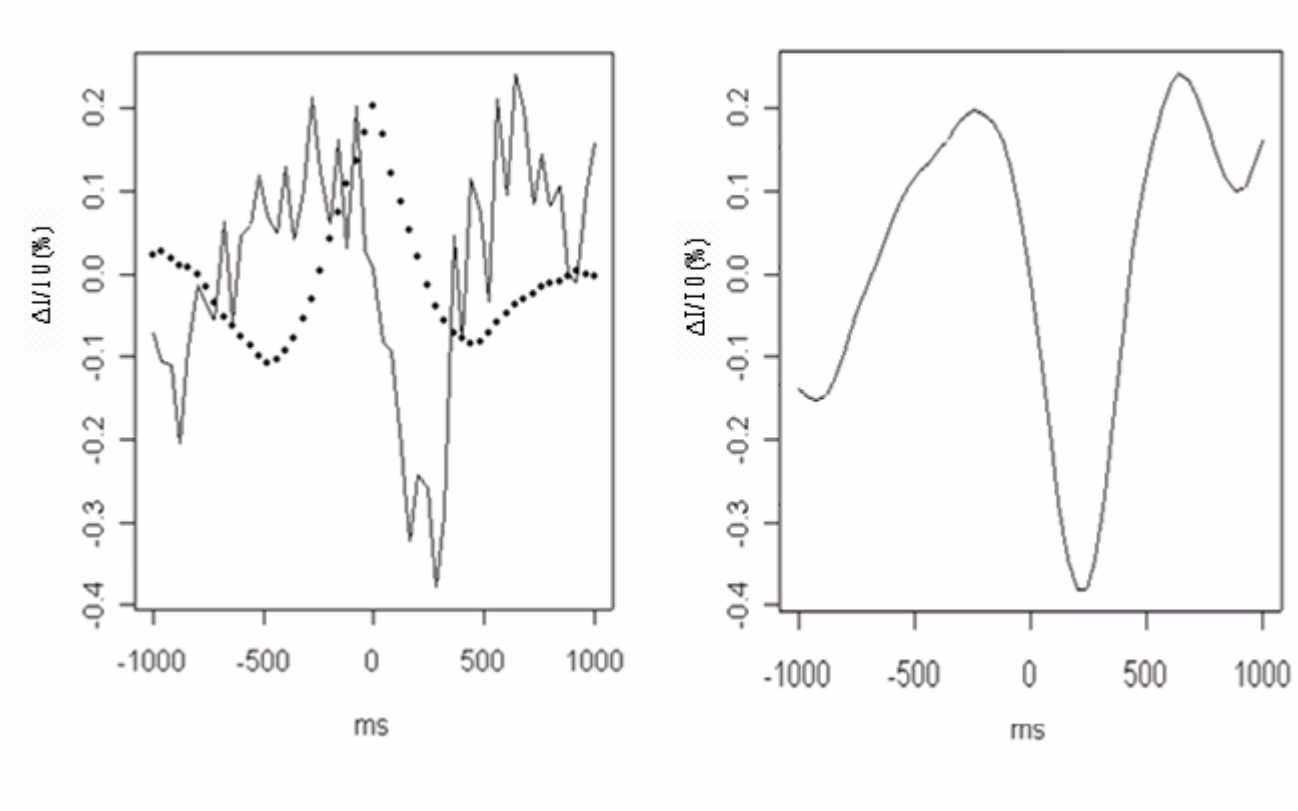
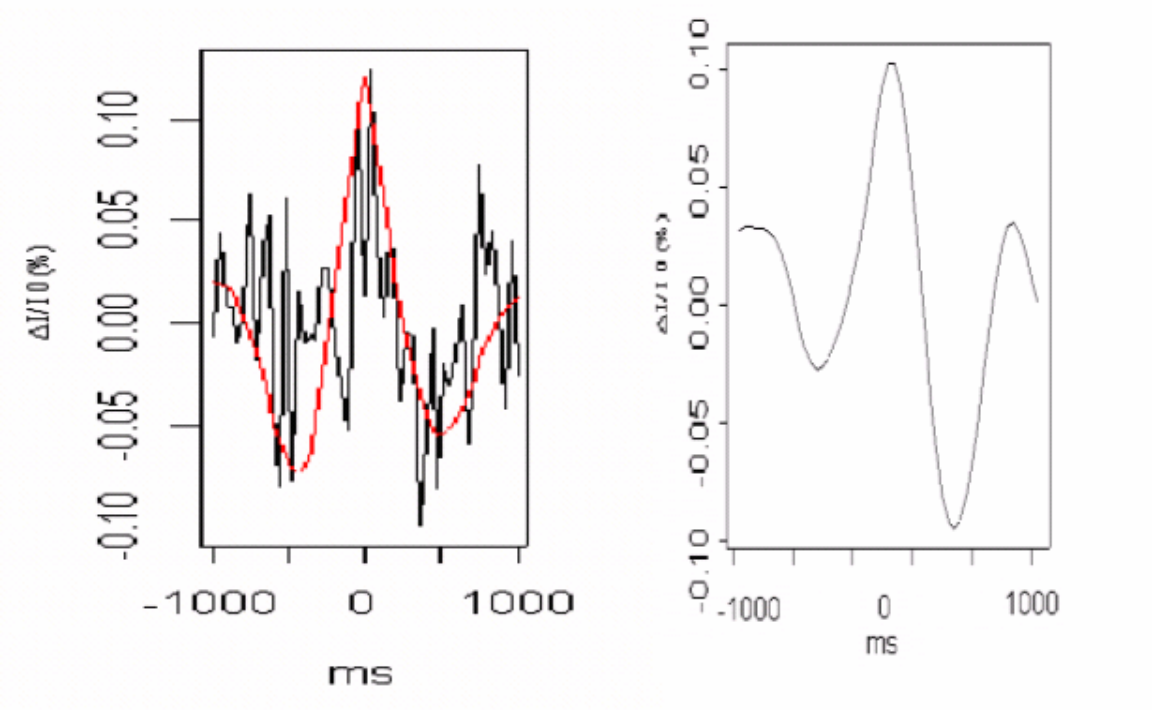
Frequency (Hz)



_____ Optical data

••••••• Electrical data



_____ Optical data
_____ Electrical data

Expanded Literature Review

Near-infrared spectroscopy (NIRS) is a relatively new technology to study the brain activity and much remains to be learned about its physiology and physics. In order to better understand the underlying mechanism of the optical signal, such as its relationships to neuronal as opposed to vascular activity, the delta waves in human sleep were investigated. The advantage of studying these waves is their very large size (75-200 μV). By contrast, in previous optical studies the evoked fast optical response has been investigated in the visual, motor, and auditory system as a correlate of event-related potentials (ERPs) that are quite small in amplitude (1-5 μV). Because this electrical activity is so small, signal averaging over many trials is required for patterns to be clearly seen, and effects on the optical signals have only been significant for grouped data (reviewed by Gratton, Fabiani, Elbert, and Rockstroh, 2003). Delta waves are much larger. These electrical brainwaves are found in deep sleep, and have a frequency between 1-3 Hz and an amplitude ranging from 75 to 200 μV. With such large amplitudes, it was easier to obtain a suitable signal-to-noise ratio for the optical signal in this experiment.

1.1 Near-infrared spectroscopy

Near-infrared spectroscopy (NIRS) is a useful technique for the investigation of biological tissues because of the relatively low absorption by water and other tissue components and the dominance of oxy- and deoxy- hemoglobin absorption in the 700-900 nm (Wray, Cope, Delpy, Wyatt, and Reynolds, 1988). Due to the overall low absorption in this band, NIR light can penetrate biological tissues. This characteristic of NIR light opens a window into the brain. However, due to the relatively high scattering

coefficient of skull and high scattering coefficients of white matter, it is difficult for near-infrared light to penetrate more than a few centimeters into the head. For this reason, NIRS essentially limited to assessing cortical function (Gratton et al., 2003; Gratton, and Fabiani, 1998).

There are different approaches to near-infrared (NIR) measurement. The frequency domain system used in this study uses light sources that are modulated at relatively high frequencies (>100 MHz) and provide two intensity measures ("AC" + "DC", discussed below) and a temporal measure ("phase" or "phase delay"). These measures are able to provide separate estimates of absorption and scattering spectra within biological tissue. Among the various approaches, frequency domain systems seem to offer the best balance of cost for measuring fast scattering effects while simultaneously providing quantitative estimates of hemodynamic responses (Maclin et al., 2003).

1.2 Optical Signals

When photons enter biological tissue, there are three major types of interactions: absorption, scattering and Doppler shifts. Absorption may lead to (1) radiationless loss of energy to the medium, (2) fluorescence, or (3) phosphorescence. In florescence; a photon is absorbed by a molecule and shortly thereafter the molecule may emit a photon which usually has a lower energy than the absorbed photon, the rest of the energy being dissipated as heat. In phosphorescence, the interval between absorption and emission of a photon by the same molecule is longer than 10-60 s. Scattering is a phenonemon in which the photon remains at the same energy level and is merely deviated in its trajectory. Doppler shifts of the scattered photons are due to moving particles in the tissue, mainly blood (Gratton, et al., 2003; Villringer and Dirnagl, 1997).

The most important endogenous absorbers in brain tissue are oxygenated hemoglobin (oxy-Hb), deoxygenated hemoglobin (deoxy-Hb), and cytochrome-C-oxidase (Cyt-Ox). Oxy- and deoxy- hemoglobin have very different absorption spectra. This makes it possible to measure changes of concentration of oxy- and deoxy-hemoglobin using a spectroscopic approach (Gratton, et al., 2003; Villringer et al., 1997). Jobsis (1977) showed that one can detect changes in oxy- and deoxy- hemoglobin non-invasively by examining light transmission at different wavelengths.

Hemoglobin oxygenation levels change within a few seconds after the activation of a particular cortical area. There are several different optical correlates of such changes following a brief stimulation. First is the so-called "fast optical signal". This response has been observed at a latency between 60 and 160 ms when the participant was given electrical median nerve stimulation at the wrist, or with a latency about 40 ms when given checkboard visual stimulation (Wolf et al., 2003; Steinbrink, Kohl, Obrig, Curio, Syre, Thomas, Wabnitz, Rinneberg, and Villringer, 2000). When fMRI (functional magnetic resonance imaging) and optical recordings were colocalized in human subjects, this optical intensity change was highly correlated with the initial dip signal of fMRI, which reflected a rapid increase in deoxyhemoglobin due to a brief period of anaerobic metabolism (Cannestra, Pouratian, Bookheimer, Martin, Beckerand, and Toga, 2001; Malonek, and Grinvald, 1996). However, the fast optical signal is more highly localized than the subsequent hemodynamic changes, so it must have a different origin. According to Cohen (1973) and Rector (1995), the fast signal could originate from action potentials and consequent swelling of the neuronal cells. Villringer (1997) found that this physiological event was soon followed by a decrement in deoxy-hemoglobin

concentration and increase of oxy-hemoglobin concentration (at least between approximately 5 seconds and 60 seconds after the onset of brain activation).

Scattering in brain tissue originates from light travelling through regions of mismatched refractive indices. Within brain tissue, there are boundaries between intra- and extracellular space and between organelles within the cell (Villringer et al., 1997). It had been well documented that light scattering is associated with neuronal activity, and this had been attributed to reorientation of molecules in the membrane or to volumetric changes of the cell (Cohen, 1973; Hill, and Keynes, 1949; MacVicar, and Hochman, 1991). Several studies (e.g.,Gratton et al., 2003) have suggested that neural tissue generates more scattering when it was inhibited and less scattering when it was activated.

As Gratton (1998) defined photon delay, it is the time taken by photons to travel through the head from the light source to the detectors. This delay can be expressed in one of three ways in a frequency-based system: It can be expressed (1) as phase delay (in degrees of angle) of the modulated signal emerging from the head with respect to the input signal, or with respect to a baseline level; (2) as time delay of the signal (in picoseconds, 1ps= 10^{-12} sec), which can be directly derived from the phase-delay measure; or (3) as a statistical transformation (e.g., t scores or z scores) of one of the other two measures.

Neuroimaging based on photon delay was first described by Gratton's group in 1995. For this measure they coined the term EROS, or Event-Related Optical Signal. EROS has been studied in different cortical systems: visual, motor and auditory. EROS was shown to be a fast optical signal with a latency of 100 ms in occipital areas in response to the visual grid stimulation (Gratton, Corballis, Cho, Fabiani, and Hood,

1995). A tapping experiment showed that changes in the EROS can be observed at the hand movement frequency (0.8 Hz) and its harmonics (1.6 Hz, 3.2 Hz, etc). The signal was larger over the contralateral hemisphere for hand tapping but showed a smaller degree of lateralization for foot tapping. This could be explained by the fact that primary motor areas associated with the feet are located on the mesial surface between the two hemispheres (Gratton, G., Fabiani, Friedman, Franceschini, Fantini, Corballis, and Gratton, E., 1995). Gratton, Fabiani, and DeSoto (1998) recorded ERPs and EROS simultaneously over the motor cortex. Their data showed that both electrical lateralized readiness potentials and EROS signals peak at about 300 ms, which was the average reaction time for the subject. In a recently published article, Gratton et al. (2003) replicated and extended the EROS data for visual cortex as reported in their 1995 paper. The EROS response in the experimental condition peaked at a latency of 100 ms and showed a secondary peak of minor amplitude at a latency of about 300-400 ms. The short latency of these effects suggest that EROS reflected neuronal activity rather than hemodynamic activity of the brain.

1.3 Comparison of Neuroimaging Method

There are several functional brain imaging methods for investigating brain activity. The most common imaging techniques can be classified into two groups, hemodynamic and electrical. Hemodynamic methods (positron emission tomography, PET; single photon emission computed tomography, SPECT; & functional magnetic resonance imaging, fMRI) have excellent spatial resolution, in that they can delimit regions of increased or decreased neural activity with great precision (< 1 cm, in the case of fMRI). However, these techniques are limited in terms of their temporal specificity,

mainly because hemodynamic effects develop slowly, with onset occurring about 2 sec after a brain area becomes active and peak occurring 5-10 sec thereafter (Gratton et al., 1998). This is too slow to distinguish the ordering of different cognitive processes.

In contrast with hemodynamic methods, EEG-based methods have excellent temporal resolution (< 1 ms) but poor spatial resolution. The main problem with localizing the source of EEG potentials is that electrical activity in any given part of the brain is volume conducted throughout the entire head. Distortion due to source geometry, variations in resistance, and multiple active sites is severe. Although modern, computer-based algorithms for dipole localization can provide plausible hypotheses, there is always infinite number of possible solutions to the problem of localizing an activated brain area. Thus, it is still difficult to determine which structures contribute to the signal.

It would be particular useful to have a technique with combined high spatial and temporal resolution to study psychophysiological processes. Thus the association between particular brain areas and the occurrence of a particular psychological event could be easily investigated (Gratton et al., 1998). It has been noted that EROS has an advantage over other techniques for studying neuronal processes in that it potentially combines good temporal resolution (at the level of milliseconds, which is similar to EEG) with a spatial resolution in the sub-centimeter region (as good as PET, not quite as good as fMRI) (Filiaci, 2001).

Although promising in these regards, EROS has its own limitations. At least with regard to the current version of the system, there are two major concerns. First, the signal-to-noise ratio is poor. Informal comparison with EEG-based measures of visual or motor activity (e.g., DeSoto, Fabiani, Geary, and Gratton, 2001) suggested that the S/N

ratio is at best about half as good for EROS. Therefore, at least four times as many trials per condition are required to obtain signal-averaged data of similar quality. A second problem is that the current system can only record at shallow depths, limiting its range of view to superficial neocortical structures. The limitation in penetration is due to the attenuation of light with distance in the head. If both the detector and source were on the same surface, the penetration of the method is estimated at a maximum of about 5 cm (Gratton, Fabiani, and Corballis, 1997). Our own measurements, using an animal preparation, suggested even less penetration. With the light source positioned inside the brain of a dead calf and the detector at the surface, we documented a reliable penetration to about 3 cm beneath the interior surface of the skull.

2. Sleep

2.1 Stages of sleep:

Traditionally, there are three fundamental measures used to define the stages of sleep (Rechtschaffen and Kales,1968). Electroencephalography (EEG) records gross brain wave activity; the electro-oculogram (EOG) measures eye movement; and muscle tone is recorded via electromyography (EMG). The combined recording of these and other measures of bodily activity during sleep is termed “polysomnography” (PSG).

During a normal night’s sleep, there are alternating rapid eye movement (REM) and non-REM (NREM) stages of sleep (Pace-Schott and Hobson, 2002). REM sleep is characterized by a sudden and dramatic loss of muscle tone, as measured by EMG. The EEG is similar to that of waking, mainly desynchronized, but with occasional low-amplitude bursts of beta (15-30 Hz) and alpha (8-12 Hz) waves. The longest duration and most intense dreaming happens in REM sleep (Hobson and McCarley, 1977; Hobson,

Pace-Schott, and Stickgold, 2000). Non-REM sleep is further divided into 4 stages. Stage 1 is characterized by theta waves (4-8 Hz), alpha waves, and desynchronized EEG. Stage 2 sleep has electrical transients referred to as sleep spindles and K complexes, and small amounts of delta waves (0.5-4 Hz). Sleep spindles are short bursts of waves at 12-15 Hz. K complexes are sudden, sharp negative-then-positive waveforms, which are found mainly during Stage 2 sleep. Stage 3 is defined by the presence of delta waves that occur less than 50 percent but more than 20 percent of the time. Finally, Stage 4 has more than 50 percent delta waves. Stages 3 and 4 also are referred to as slow wave sleep (SWS) because of the presence of delta activity (Horne, 1988). During a normal night's sleep, people alternate between periods of REM and non-REM sleep. Sleep begins in Stage 1 progresses to Stage 4, then back through the stages, with the exception that Stage 1 is replaced by REM. Then the sleeper goes back to Stage 2 and so on. Each cycle is approximately 90 minutes, which contains about 10-20 minutes of REM sleep (Hartmann, 1967).

2.2 Mechanisms of generation of delta waves:

Delta waves are thought to have a dual origin: They can be either generated within the cortex alone or generated between the cortex and thalamus, through an interaction involving intrinsic thalamic cell-membrane currents. Cortico-thalamic generated delta waves may result from activation of inhibitory interneurons which hyperpolarize the thalamocortical cells (Steriade, Contreras, Curro, and Nunez, 1993; Hobson and Pace-Schott, 2002). When activation of the thalamocortical system by ascending arousal systems is withdrawn, spontaneous cortical slow oscillatory activity triggers and synchronizes EEG spindles and delta waves. Sleep spindles (sigma

frequency, 12-15 Hz), delta waves and K-complexes are all characteristic waveforms of NREM sleep EEG.

2.3 Functional neuroimaging of sleep:

PET studies showed that in slow-wave sleep the most deactivated areas (relative to waking) include the dorsal pons and mesencephalon, cerebellum, thalamic nuclei, basal ganglia, basal forebrain/ hypothalamus, prefrontal cortex, anterior cingulate cortex, precuneus and the mesial aspect of the temporal lobe (Maquet, 2000). During rapid-eye movement sleep, significant increases in activity (relative to NREM) were found in the pontine tegmentum, thalamic nuclei, limbic areas (amygdaloid complexes, hippocampal formation, anterior cingulate cortex) and in the posterior cortices (temporo-occipital areas). During NREM sleep, there were global cerebral energy metabolism and blood flow decreased compared with waking and REM sleep (Maquet, 2000). With the greater depth of NREM sleep, the energy metabolism decreased even more. By contrast, global cerebral energy metabolism during REM sleep was equal to or greater than that which occurs during waking (Maquet, 1995; Hobson et al., 2002). Functional magnetic resonance imaging had also confirmed that the cerebral metabolic rate of oxygen was practically the same during REM sleep as during wakefulness, whereas a 23 percent decrease was found during Stage 2 and 3 for NREM sleep (Lovblad, Thomas, Jakob, Scammell, Bassetti, Griswold, Ives, Matheson, Edelman, and Warach, 1999). Activation of occipital cortex and deactivation of frontal cortex during REM sleep was observed, which is in agreement with previous PET studies. Hobson (2002) noted that decreased blood flow in the thalamus and in the prefrontal and multimodal parietal cortices

accompanied the onset and deepening of NREM sleep. He suggested that decrease of blood flow in the thalamus corresponded to generation of delta waves.

3. NIRS and Sleep

Historically, there had been a keen interest in investigating the electrophysiology and functional significance of human sleep. NIRS has also been applied to study sleep both in human beings and in monkeys. Continuous recording by near infrared spectroscopy was performed in monkeys during the normal sleep-wake cycle by Tamura and colleagues (1997). They suggested that non-REM sleep stage (Stages I, II, III) did not have varied oxygenation state as compared to each other. By contrast, during REM sleep, there was increased cerebral oxygenation to a level even higher than that in the conscious state. The oxygenation level decreased rapidly when the monkeys fell to non-REM sleep again. When applying NIRS to human beings, studies (Tamura et al., 1997) have also indicated that during the first transition from the awake stage to non-REM sleep, subjects showed decreases in both oxygen consumption and blood-flow. During REM sleep, cerebral oxygenation state increased with an increase of blood flow, but did not exceed the level of the awake state. However, this study also found that several volunteers showed increases in oxygen consumption in the first transition, that is from awake state to non-REM sleep. The mechanism given to explain this phenomenon was that switches in the brain tissue during the first transition from wakefulness require energy. But once the brain was well into the sleep-cycle, decreased neural activity in non-REM sleep leaded to the decreased oxygen consumption, as compared to waking.

Spielman (2000) studied the transition between wakefulness and sleep using near-infrared spectroscopy. In contrast to Tamura et al., he found that cerebral metabolism was

gradually reduced at the beginning of sleep. Oxygenated hemoglobin and deoxygenated hemoglobin in frontal areas were reduced rather than increased during the wakefulness to sleep transition in human beings. At sleep offset the oxygenated hemoglobin and deoxygenated hemoglobin significantly increased. All these characteristics suggested decreased neuronal activity, reduced oxygen consumption, and reduced cerebral blood flow and volume during NREM sleep.

Another study (Rector, Poe, Kristensen, and Harper,1995) reported the hippocampal light reflectance relationships to electroencephalographic patterns during sleep in cats. They found that reflectance changes occurred in a similar frequency range as EEG changes. Peak spectral frequency for light reflectance (about 5.6 Hz) was similar to the higher EEG frequency peak (about 5.5 Hz) of the two slow activity bands during REM sleep, but not the lower frequency (peaking at about 4.9 Hz). Additionally, 4-6 Hz frequencies appeared in the reflectance signal during quite sleep (i.e., NREM) as well as rapid eye movement sleep (REM). Since none of the potential vascular influences change sufficiently fast to accommodate frequencies as high as 12 Hz in this study (the highest rate of vascular change would be 0.5 Hz), these reflectance changes indicated that fast acting processes, such as cellular swelling or protein conformation changes, might be the underlying mechanisms.

However, these rhythmic slow waves appeared similar in the reflectance signal during quiet sleep and rapid eye movement sleep, which showed quite different power spectra in EEG. It was not safe to say the optical reflectance signals really corresponded to the patterns of the EEG. Note also that the subjects were cats rather than human beings in their study. Furthermore, none of the existing studies have clearly illustrated the

relationship between delta waves in EEG and optical recordings. Since delta waves have a relatively high amplitude (over 75 μV), they provide a good chance to find a correspondence between electrical and optical recordings in individual participants.

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Consent Form

1. I hereby consent to take part in research conducted by Drs. Steve Hackley and Jun Zhao at the Cognitive & Clinical Neuroscience Lab of the University of Missouri. I understand that other persons may assist them or be associated with them.

2. It has been explained to me and I understand that:

   A. The purpose of this research is to investigate optical neuroimaging of human sleep. Optical neuroimaging in this study will let us see what your brain is doing while you sleep. While you take a nap, we will shine infrared lights in your brain. By seeing what happens to those lights we will be able to understand electrical activity in the brain that occurs during sleep.

   B. My participation will consist of taking a nap. During my nap, the electrical activity of my brain, eye, and muscles will be recorded with electrodes attached to the skin, and optical images will be obtained with sensors on my forehead. I understand that the naturally occurring electricity in my body will be measured, but no electricity will be applied to my body. If I am unable to fall asleep or do not stay sleep for the required length of time, I will nonetheless be granted full credit for my participation. This 4-hour long experiment will take place in Psych Building Room 110.

   C. There is no reasonable basis for expecting my participation in this research to expose me to the risk of harm or serious discomfort. However, it is necessary to rub the skin at places where electrodes will be attached. Some subjects may experience a reddening, tenderness, or even abrasion at these sites. If this occurs--which is quite rare--it will disappear in a few days. In previous studies 10% of participants developed headaches as a result of their participation. However the new methods used in the present study should reduce that risk. Please contact Dr. Zhao (884-6940) or Dr. Hackley (882-3277) if any skin irritation or headache persists. I will be told of any significant new information that might affect my willingness to take part in this research.

   D. There is no other way to get the information required for this research.

   E. This experiment will likely lead to advances in biomedical knowledge that will benefit humans and animals in general, but are unlikely to be of special benefit to me, individually. If unexpected medical information is discovered, we will contact you promptly.

F. My participation is strictly voluntary; I may end my participation at any time.

G. If I do not volunteer to take part in this research, or if my participation is ended for any reason by the researchers or me, this will have no effect on any care, treatment, or other benefits for which I am entitled. My grades will not be affected if I chose not to participate or if I withdraw at any time during the study.

H. The results of this research may be published, but I will not be identified in any such publication. My research data and clinical records will be treated as confidential.

I. By signing this form, I indicate that any questions I have had up to this point in time have been answered. If I have any further questions, I am to contact Dr. Zhao or Dr. Hackley, 573-884-6940 (Room 112, Psych Building). Or If I have further question about my rights as a participant, I can contact the Campus IRB 573-882-9585 (483 McReynolds) to get more information.

3. I further consent to allow Drs. Zhao and Hackley and their associates to perform the procedures referred to; to report their findings to government agencies, funding agencies, manufacturers, or scientific bodies; and to publish their findings.
4. I will receive either course credit or a small payment as partial compensation for my time upon completion of the experiment. I am aware that my participation in this study will at least partially fulfill the research requirements for my Psychology 1 class. I am also aware that there are alternative ways of fulfilling my research requirement (e.g., completing a short paper; completing an exam on alternative readings). These alternatives are described in the syllabus for my Psychology 1 class.

Signature: _____ Date: _____

General Briefing

In this experiment the subject's brain waves are recorded while he/she is taking a nap. We are especially interested in the brain waves that occur during deep sleep.

The main purpose of this experiment is to better understand the underlying mechanism of one kind of neuroimaging technique---optical neuroimaging. Optical neuroimaging in this study will let us see what your brain is doing while you sleep. While you take a nap, we will shine infrared lights in your brain. By seeing what happens to those lights we will be able to understand electrical activity in the brain that occurs during sleep. We are particularly interested in observing Delta waves, one kind of electrical activity found in deep sleep.

If you have any additional questions regarding this experiment please feel free to ask the experimenter about it. I am aware that my participation in this study will at least partially fulfill the research requirements for my Psychology 1 class. I am also aware that there are alternative ways of fulfilling my research requirement (e.g., completing a short paper; completing an exam on alternative readings). These alternatives are described in the syllabus for my Psychology 1 class. Thank you again for your participation.