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Spontaneous Low Frequency Oscillations of Cerebral Hemodynamics and Metabolism in Human Adults

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We investigated slow spontaneous oscillations in cerebral oxygenation in the human adult's visual cortex. The rationale was (1) to demonstrate their detectability by near infrared spectroscopy (NIRS); (2) to analyze the spectral power of as well as the phase relationship between the different NIRS parameters (oxygenated and deoxygenated hemoglobin and cytochrome-oxidase; oxy-Hb/deoxy-Hb/Cyt-ox). Also (3) influences of functional stimulation and hypercapnia on power and phase shifts were investigated. The results show that—in line with the literature—low frequency oscillations (LFO) centred around 0.1 s^{-1} and even slower oscillations at about 0.04 s^{-1} (very low frequency, VLFO) can be distinguished. Their respective power differs between oxy-Hb, deoxy-Hb, and Cyt-ox. Either frequency (LFO and VLFO) is altered in magnitude by functional stimulation of the cortical area examined. Also we find a change of the phase shift between the vascular parameters (oxy-Hb, tot-Hb) and the metabolic parameter (Cyt-ox) evoked by the stimulation. It is shown that hypercapnia attenuates the LFO in oxy-Hb and deoxy-Hb. Conclusions: (1) spontaneous vascular and metabolic LFO and VLFO can be reproducibly detected by NIRS in the human adult. (2) Their spectral characteristics and their response to hypercapnia are in line with those described in exposed cortex (for review see (Hudetz *et al.*, 1998)) and correspond to findings with transcranial doppler sonography (TCD) (Diehl *et al.*, 1995) and fMRI (Biswal *et al.*, 1997). (3) The magnitude of and phase relation between NIRS-parameters at the LFO may allow for a local noninvasive assessment of autoregulatory mechanisms in the adult brain.

INTRODUCTION

Slow oscillations of cerebral hemodynamics and metabolism have been studied by different techniques and in different species (Table 1). Their origin is unclear, their frequency spectrum is ill defined and the terminology confusing since vasomotion or V-signal, spontaneous oscillations, and low frequency waves are used synonymously, in part not respecting gross differences in frequency in species and in parameters monitored. Beyond these incongruencies the following features are common to the phenomena described: they occur without any overt stimulus (spontaneity), they can be differentiated from other oscillatory phenomena such as the heart beat and respiratory cycles (slowness), and they are altered by pharmacological and pathological conditions (modulatability).

Besides the interest in the underlying mechanisms, the oscillations have lately gained interest for three reasons. (1) Functional connectivity maps have been reported on their basis (Biswal *et al.*, 1997; Lowe *et al.*, 1998). (2) TCD (transcranial doppler sonography) studies have shown that their phase relation to aBP-oscillations of the same frequency may reveal autoregulatory mechanisms of the brain vasculature (Hu *et al.*, 1999; Diehl *et al.*, 1995). (3) Alterations due to cerebral ischaemia have been shown in a rat stroke model (Mayevsky and Ziv, 1991).

The purpose of the present study was to investigate these oscillations in the healthy human adult with near infrared spectroscopy (NIRS), a noninvasive tool to measure cortical hemodynamics and metabolism. The goal is to find out in how far the slow spontaneous oscillations noninvasively monitored in the human cortex may play a role in functional activation research and in the monitoring of cerebrovascular pathology.

The first step toward this goal is to establish that NIRS allows for the noninvasive detection in the adult. There have been previous reports using NIRS in neonates (Livera *et al.*, 1992). Concerning the human adult Elwell and coworkers (Elwell *et al.*, 1999) just recently published a study on the oscillations in question, which has so far been the only systematic approach with a sound analysis of amplitude and frequency spectrum. Other previous reports analyzed data with respect to patterns, which were highly variable across subjects (Hoshi *et al.*, 1998; Hoshi and Tamura, 1997). A pioneering work by Chance *et al.*

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TABLE 1

Selection of Articles on Spontaneous Low Frequency Oscillations of Cerebral Blood Flow and Metabolism

Authors	Ref.	Species	Technique	Parameter	VLFO/s^{-1}	LFO/s^{-1}	HFO/s^{-1}	Magn.	Modulation	Remarks
Kleinfeldt	1995	rat	2-Photon microsc.	RBCv		~0.1				Cohere for <0.1 Hz
Biswal	1996	rat	Video-microscopy	RBCv		0.07–0.2			L-NAME	
Dirnagl	1993	rat	LDF	CBFv		0.1–0.18		18%	NOS-inhibitor	
Golanov	1996	rat	LDF	CBFv		~0.1		20%	Spontaneous & evoked by identified neurons	
Golanov	1994	rat	LDF	CBFv	~0.1		20%	Anesthesia		Synchron. across brain
Hudetz	1995	rat	LDF	CBFv		0.11–0.13		10%	L-NAME, CO_2, anaesth.	
Hudetz	1992	rat	LDF	CBFv		0.07–0.18		14–30%	RR, CO2	
Morita	1992	rat	LDF	CBFv		0.08–0.17		5–10%	RR, U-shaped dependence	Frequency shifts by RR
Dora	1981	cat	Fluororeflectometer	NAD/H, CBV	0.03–0.05	0.08–0.17				Lag of NADH
Mayevsky	1991	rat	Fluororeflect./LDF	NAD/H, CBV, CBFv		0.1–0.15			CO_2, O_2	Ischemia related
Vern	1988	cat	Reflect.-spectrosc.	CYT, CBV		~0.15			No by sleep	Indep. metab./ vasc.
Vern	1997	rabbit	Reflect.-spectrosc.	CYT, *CBV*		<0.5		2–5%*[3]		Connectivity
Mayhew	1999	rat	Spectrosc. imaging	*HbO2, Cyt*		~0.1		5× stim.*[1]*[3]	Stimulation?	Phase shift HbO2-Cyt
Mayhew	1996	rat/cat	Spectrosc. imaging	Intrinsic signal		~0.1		1–2%	Stimulation?	Temp/spat inhomogen.
Cooper	1966	human	Polarogr. electr.	Invasive: pO_2		~0.1		−20%	Hypercapnia	Spatial inhomogen.
Livera	1992	neonate	NIRS	tot-Hb		0.05–0.08		(~3 μM)	Pathology?	
Chance	1993	human	NIRS	Absorption		0.1–5			Stimulation?	
Ebwell	1996	human	NIRS	*oxy*-, deoxy- & tot-Hb			~0.2	0.4 μM*[2]*[3]		
Ebwell	1999	human	NIRS	*oxy*-, deoxy- & tot-Hb		0.08	0.22	0.6 μM*[2]*[3]		
Hoshi	1998/7	human	NIRS	oxy-, deoxy- & tot-Hb	0.01−	0.08				
Diehl	1991/5	human	TCD	MCA-Fv	0.007−	0.15		−30%	Phase by ICA occlusion	Phase shift RR-LFO
Giller	1999	human	TCD	MCA-FI	0.006–0.037			7.5%		
Hu	1999	human	TCD	MCA-Fv	0.016–0.04	0.04–0.15	0.15–0.4		Phase/magn. by ICA occlusion	Corr. with CO_2-test
Kuo	1998	human	TCD	MCA-Fv	0.016–0.04	0.04–0.15	0.15–0.4			Phase aBP-Fv
Zhang	1998	human	TCD	MCA-Fv	<0.07	~0.1	~0.2		Cerbral vasculature functions as high pass filter	
Zhang	1998	human	TCD	MCA-Fv	0.02–0.07	0.07–0.2	0.2–0.3	Orthostatic stress		Indicator of autoreg.
Bäzner	1995	human	TCD	MCA-Fv	0.01–0.05	0.05–0.15	0.15–0.5		Large/small artery disease	
Blaber	1997	human	TCD	MCA-Fv	~0.03	~0.1	~0.2		Orthostatic stress	Autoreg. = high pass filter
Mitra	1997	human	BOLD-fMRI	BOLD-contrast		~0.1				Temp/spat inhomogen.
Biswal	1997	human	BOLD-fMRI	BOLD-contrast	~0.02	(~0.14)			Hypercapnia	Connectivity
Biswal	1995	human	BOLD-fMRI	BOLD-contrast		<0.08				Connectivity
Lowe	1998	human	BOLD-fMRI	BOLD-contrast		<0.08				Connectivity
Li	2000	human	BOLD-fMRI	BOLD-contrast	~0.04	(~0.1)	~0.23		Cocaine	Connectivity

Note. The list is incomplete and based on the articles cited in the text. For convenience of comparison frequencies were converted into s^{-1}, if the original papers report cycles per minute or wavelength. The magnitude (column 9) is given in % of the baseline value. *[1]: magnitude in relation to the response elicited by a stimulation. *[2]: μM change assumes DPF of 6. *[3]: change in the parameter given in italics in column 4. LDF: laser-doppler-flowmetry; TCD: transcranial doppler sonography; RBCv: red blood cell velocity; CBFv: cerebral blood flow velocity; CBV: cerebral blood volume; CYT: cytochrome-oxidase redox state as measured by dual wavelengths spectroscopy; HbO2, Cyt corresponding to oxy-Hb and Cyt-ox, measured, however, in the visible spectrum; MCA-Fv/MCA-FI: middle cerebral artery flow velocity/Index. Only the first authors are given. Bracketed frequencies are taken from figures.

(1993) investigates a broad range of frequencies from 0.1–5 s^{-1} in response to physiological stimulation. Besides the alteration of the power of the oscillations during hypercapnia and functional stimulation of the cortical area illuminated, the analysis was focused on phase shifts between NIRS parameters. The method has been shown to monitor both vascular ([oxy-Hb]/[deoxy-Hb]) and metabolic ([Cyt-ox]) changes in the cortex (Jobsis, 1977; Cooper *et al.*, 1997; Heekeren *et al.*, 1999). Thus shifts between the parameters may correlate to alterations in the interplay of cerebral blood flow velocity (CBFv), cerebral blood volume (CBV), and metabolic demand of the cerebral tissue. To noninvasively monitor spontaneous oscillations in [deoxy-Hb], [oxy-Hb], and [Cyt-ox] may further the link between the numerous invasive optical animal studies (Vern *et al.*, 1997; Hudetz *et al.*, 1998; Mayhew *et al.*, 1996) to similar studies using fMRI (Biswal *et al.*, 1997; Mitra *et al.*, 1997) and TCD (Giller *et al.*, 1999) in the human.

METHODS AND ANALYSIS

Twenty-one experiments were performed in 17 subjects (14 ♀, 3 ♂, age range 23–32 years.). Some subjects participated in all three experiments. The subjects had no history of neurological or vascular disease, gave informed consent to the study, which was agreed on by the Ethics Committee of our hospital and were financially rewarded for their participation.

Experiment 1

Thirteen subjects underwent visual stimulation with an annular checkerboard alternating at 8 s^{-1} (full-field stimulation) presented on a computer screen. Resting condition consisted of the presentation of a dark grey screen while fixation was maintained. The stimulus has been shown to evoke a vascular response in the primary visual cortex in PET (Vafaee *et al.*, 1999), fMRI (Kruger *et al.*, 1998), and NIRS (Heekeren *et al.*, 1997). Each stimulation period lasted 120 s and was preceded by an equally long resting period. Five cycles were performed in each block of experiments and all subjects underwent two or three blocks in one session. The probe positioning was guided by external bony landmarks (inion) (Steinmetz *et al.*, 1989) and avoided the midline, for reason of the underlying saggital sinus. In many subjects MRIs, performed previously, helped to optimise probe position over the calcarine fissure.

Experiment 2

Four subjects were examined with the same protocol (three blocks of five cycles each), however, between the first and second block a hypercapnia was induced by inspiration of 5% CO_2 (in 21% O_2 and 74% N_2). The second block was started 3–5 min after the onset of breathing 5% CO_2. At the end of the second block subjects again breathed room air and following a 3- to 5-min interval the last block of five stimulations followed. In three of these subjects arterial blood pressure (aBP) was coregistered with the noninvasive finger plethysmographic method (Finapress, Ohmeda, U.S.A.).

Experiment 3

Four subjects were examined not undergoing any stimulation. In these subjects two detector probes were fixed to the subjects occiput, one at 0.5 cm and the second at 3.0 cm from the light emitting probe. The rationale was to sample from two different layers of tissue. With the small distance the changes in oxygenation monitored are dominated by changes in the skin and skull. The larger probe separation is expected to also sample deeper layers, i.e., the cerebral cortex (see Fig. 7a). Subjects were monitored for at least 25 min. During the last 5 min subjects were instructed to breathe at 0.1 s^{-1}. In- and expiration were guided by acoustic cues.

For all experiments subjects were seated in an EEG-chair in a quiet dimmed room. The probes were protected from ambient light by black cloth.

Technical Set-Up

The NIRS measurements were performed in continuous wave mode allowing for assessment of changes in chromophore concentration along the theory of Jobsis (1977). In brief under assumption of a constant optical pathlength the changes in light attenuation (optical density) at different wavelengths are ascribed to changes in chromophore concentration in the illuminated tissue. The applicability for functional cortical activation studies has been demonstrated by a number of investigators (for review see (Obrig and Villringer, 1997)). For experiment 1 and 2 a single emitter single detector CCD approach was used as previously described (Heekeren *et al.*, 1999; Kohl *et al.*, 1998). A halogen bulb (700–1000 nm) serves as light source and a CCD camera (1024 × 256 pixels; Princeton Instruments, SI, Germany) in conjunction with an imaging spectrograph (SP-275, Acton Research) as detector. For experiment 3 the light detected by the two detector probes is projected on different segments of the CCD chip. By separately reading out the two segments the two channels can be measured simultaneously. To maximize light intensity we used a slit width of 1.0 mm. This leads to a blurring of the collected spectra. To compensate for the effect the reference spectra were also smoothed over the effective spectral resolution (20 nm, FWHM). The procedure has been shown to sufficiently separate changes in [oxy-Hb], [deoxy-Hb], and [Cyt-ox] (Heekeren *et al.*, 1999). For off-line analysis the spectra were stored from 700–1000 nm. For exper-

iment 1 and 2 sampling rate was 10 s^{-1} (i.e., 12,000 spectra were acquired for each block and subject). For the third experiment the sampling rate was reduced to 3.8 s^{-1}, since faster sampling rates increase the spillover of light between the different sectors of the CCD camera, thereby blurring differences between the two probe positions. With the current set-up this blurring effect would be maximally 4% (11 ms read-out time with 265 ms total exposure time for the CCD chip).

Arterial blood pressure (aBP) was monitored by a Finapress monitor (Ohmeda, U.S.A.). Since changes rather than absolute values were of interest the automatic calibration was switched off, to allow continuous recording. Therefore aBP results are reported in arbitrary units (Jones *et al.*, 1992). The blood pressure was further assessed with an arm cuff before and after the experiment. None of the subjects showed signs or had a history of hyper- or hypotonia. ECG and respiration were also monitored and all systemic parameters were coregistered with Spike2 (Cambridge Electronic Design). The latter two parameters were recorded for the monitoring of the subject only. For any comparison between the NIRS parameters and the aBP, the aBP values were averaged according to the recording frequency of the CCD camera (10 s^{-1}) prior to further analysis.

Analysis

(1) From the recorded spectra the concentration changes in [oxy-Hb], [deoxy-Hb], and [Cyt-ox] were calculated by a three-component least square fitting procedure (Kohl *et al.*, 1998) based on the extinction coefficient spectra of oxy-Hb, deoxy-Hb, and difference spectrum of Cyt-ox. The changes are reported in μM, based on an assumed differential pathlength factor (DPF) of 6 (Duncan *et al.*, 1996). Reference spectra were smoothed according to the effective spectral resolution (20 nm FWHM) prior to the fitting procedure. For some of the further analyzes changes in [tot-Hb] (sum of the haemoglobins) and mean intensity (ΔI_m) across the spectral range, expressed as percentage of the mean intensity across the recording, are also reported (Table 2).

(2) The grand average across all subjects was calculated after linearly detrending the individual averages (Fig. 1). For each individual block *t* statistics were performed for the difference between resting and stimulation periods omitting the first 20 s of either resting and stimulation period (paired *t* test, one sided, $\alpha = 0.05$, 4 degrees of freedom (*df*), see Table 2). Also a *t* test across the means of the individual subjects (paired, one sided, $\alpha = 0.05$, 11 *df*) was calculated (Table 2, last line).

(3) For further analysis in the frequency domain, epochs of 1024 data points (i.e., 102.4 s) were defined at the end of the stimulation and resting periods. Since the spontaneous oscillations are the issue of the present study we thereby intended to exclude the slow stimulus response. The underlying assumption is, that the resting state and stimulation are stable conditions 18 s after the onset or cessation of the stimulus. To further remove baseline shifts each individual epoch was detrended by a 3rd order polynomial (similar to studies using TCD (Zhang *et al.*, 1998a)). In these epochs the following analyzes were performed for all parameters:

(3a) Assessment of power spectral density (PSD);

(3b) Estimate of coherence ($COH^{index1 \rightarrow index2}$) between oxy-Hb → deoxy-Hb, oxy-Hb → Cyt-ox, tot-Hb → Cyt-ox. The indices notify the parameters compared. For the experiments in which arterial blood pressure (aBP) was monitored, the estimates of aBP → oxy-Hb, aBP → deoxy-Hb, and aBP → Cyt-ox were also calculated.

(3c) Transfer function estimate, between the same pairs of parameters. Only the phase of this transfer function ($PHA^{index1 \rightarrow index2}$) is reported.

For PSD, COH, and PHA the Welch method was used. Estimates were calculated using a Hanning-window of 512 data points with an overlap of 128 data points and the above mentioned 3rd order polynomial detrending. PSD and FFT were also calculated for a Hanning window of 1024 data points (without overlap and thereby not allowing for coherence analysis).

Means of PSD, COH, and PHA were calculated for each and across all subjects (see Figs. 3 to 8). *T* statistics (paired, 1-sided *t* test, 11 *df*, $\alpha = 0.05$) were performed between rest and stimulation periods across all subjects in experiment 1 for different spectral windows (see Results).

All analyzes were performed in MatLab 5.0 (The MathWorks Inc.) using in part predefined routines.

RESULTS

Experiment 1: Visual Stimulation

Response to the stimulus. Twelve of the 13 subjects examined showed a clear hemodynamic response to the checkerboard stimulus. The stimulus induced an increase in [oxy-Hb], a decrease in [deoxy-Hb] of about half the size, and a small but remarkably stable increase in Cytochrome-oxidase oxidation (Fig. 1; Table 2). These findings are in line with our previously published functional activation studies using NIRS (Obrig *et al.*, 1996; Wenzel *et al.*, 1996; Heekeren *et al.*, 1997). Two features of the response pattern should be noted. First, the low frequency oscillations survived the averaging procedure and can be clearly seen in the [oxy-Hb] and [Cyt-ox] trace during the first stimulation epoch. These low frequency oscillations are present in all parameters but most pronounced in [oxy-Hb] reaching up to twice the size of the stimulus induced changes in some subjects (compare Fig. 2B).

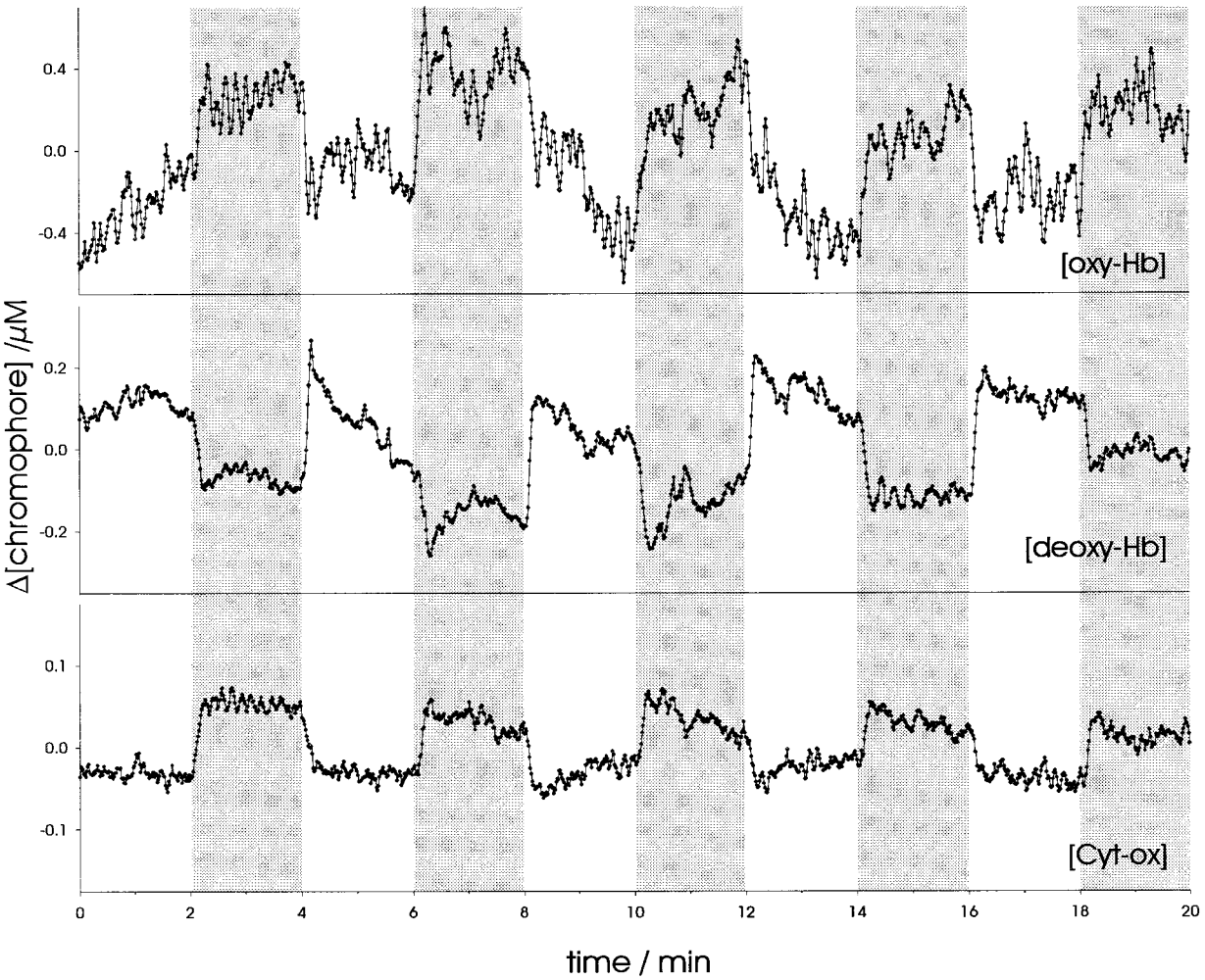


FIG. 1. Grand average across 12 subjects undergoing 5 cycles of stimulation and rest of 2-min duration each. The two or three blocks performed in each subject were averaged individually and then linearly detrended prior to the grand average. For the Fig. 10 data points (i.e., 1 s) were binned also attenuating the heart-beat induced fluctuations. Note that the slow oscillations survived the averaging procedure and are strongest in [oxy-Hb]. Changes are given in micromoles, assuming a DPF of 6 (Duncan *et al.*, 1996), with a different scaling for each parameter.

Another noteworthy feature is the initial overshoot seen in the [deoxy-Hb] recordings. There has been some controversy concerning the existence and significance of the habituation of the BOLD-contrast during prolonged stimulation in the fMRI literature. Some authors postulate a switch to oxidative metabolism over the course of 1–2 min resulting in a decrease in BOLD contrast (i.e., an increase in [deoxy-Hb]) (Frahm *et al.*, 1996; Hathout *et al.*, 1994). However, the comparison between different BOLD- and flow-sensitive MR sequences as well as different stimulus modalities (Bandettini *et al.*, 1997; Howseman *et al.*, 1998) rather supports the view that this effect is stimulus dependent and is to be accounted for by neuronal habituation. The stimulus used in the present study shows an attenuation neither in flow- nor in BOLD-sensitized fMRI over the course of 10 min. The rather short undershoot (on the order of 20 s) seen in the present experiment and the slow [deoxy-Hb] increase in some of the stimulation periods may therefore be a combination of (a) unbalanced baseline shifts, (b) a short initial overshoot due to changes in aBP, and (c) a more generalised neuronal arousal. This issue is not the focus of the present paper but is of relevance, since slow parameter shifts may produce low frequency components in the successive frequency domain analysis. As a practical consequence the epochs defined for the frequency-domain analysis omitted the first 18 s of resting and stimulation periods and were detrended with a third order polynomial. Thereafter the baseline in the epochs was stable (see Fig. 2).

Low frequency oscillations (LFO). Figures 2A and 2B illustrate two features of the low frequency oscillations, which were present in all subjects. As seen in (A)

TABLE 2

Subject		Δ[oxy-Hb] (mM)	Δ[deoxy-Hb] (μM)	Δ[Cyt-ox] (μM)	I_m (a.u.)	ΔI_m (%)
bm	1	0.075	−0.145*	0.053*	2519	0.10
	2	0.416*	−0.194**	0.072**	3429	1.27
	3	0.333$	−0.149**	0.060**	2470	1.10
	Σ	0.274	−0.163**	0.062**	2806	0.82
bs	1	0.062	−0.040	0.026**	6242	0.23
	2	0.114	−0.033	0.024**	3698	0.40
	3	0.042	−0.055$	0.022**	3972	0.07
	Σ	0.072	−0.043	0.024**	4638	0.23
sn	1	0.309*	−0.172*	0.070*	2310	0.99
	2	1.029**	−0.235**	0.069**	2949	3.37
	3	0.833**	−0.230**	0.062**	2761	2.59
	Σ	0.724*	−0.213**	0.067**	2673	2.32
so	1	0.707*	−0.179*	0.085**	2857	2.61
	2	1.139**	−0.182*	0.083**	1860	4.13
	3	0.709*	−0.234*	0.067**	2937	2.29
	Σ	0.852*	−0.198*	0.078**	2551	3.01
sr	1	0.520*	−0.137**	0.060*	2882	1.75
	2	0.106	−0.131**	0.077**	2915	0.43
	3	0.267**	−0.109**	0.064**	3277	1.06
	Σ	0.297	−0.126**	0.067**	3025	1.08
ss	1	0.226$	−0.076	0.013*	4454	0.57
	2	0.289$	−0.205*	0.017*	5167	0.23
	3	0.226	−0.028	0.010	4466	0.75
	Σ	0.247$	−0.103	0.013$	4696	0.52
st	1	0.244	−0.085	0.056*	4228	0.93
	2	0.440*	−0.160$	0.018$	4227	1.00
	3	0.081	−0.063$	0.050**	4104	0.41
	Σ	0.255	−0.103	0.041*	4186	0.78
us	1	0.417	−0.279**	0.070$	4594	0.89
	2	0.631$	−0.211**	0.043	4022	1.69
	Σ	0.524	−0.245**	0.056$	4308	1.29
wc	1	0.705**	−0.283**	0.073**	2423	1.75
	2	0.251	−0.222**	0.081**	1361	0.80
	Σ	0.478	−0.253**	0.077**	1892	1.27
am	1	0.179	−0.117**	0.010	461	0.31
	2	−0.045	−0.242	0.016	403	1.14
	Σ	0.067	−0.179$	0.013	432	0.72
js	1	0.639**	−0.387**	0.134**	1636	2.02
	2	0.722*	−0.352**	0.164**	1635	2.70
	Σ	0.680*	−0.370**	0.149**	1636	2.36
mc	1	0.919*	−0.305**	0.104**	1817	3.05
	2	0.710$	−0.203$	0.107**	1903	2.68
	Σ	0.815*	−0.254*	0.106**	1860	2.86
Mean		0.440**	−0.187**	0.063**	2892	1.44
SD		0.278	0.088	0.039	1344	0.95

Note. The table gives the individual response magnitudes for oxy-Hb, deoxy-Hb, Cyt-ox, and I_m in response to the visual stimulation. $^{**}P < 0.01$, $^{*}P < 0.05$, $^{\$}P < 0.1$ according to *t* statistics (one-sided, 4 dof for the individual blocks, 11 dof for the mean across all subjects).

there is a clear phase shift between the LFO in the different parameters. [Deoxy-Hb] changes precede [oxy-Hb] oscillations by some 2 s, while the oscillations in [Cyt-ox] lag some 4 s behind those in [oxy-Hb]. Differences between stimulation and rest are not easily visible in the time domain (compare Fig. 4). Figure 2B illustrates that the magnitudes of the oscillations differed between parameters. They were greatest in [oxy-Hb] and about 10 times smaller in [deoxy-Hb] and [Cyt-ox]. Compared to the changes induced by the visual stimulus they were about twice the size of the response in [oxy-Hb] and [Cyt-ox]. For [deoxy-Hb] they amounted to about the same size as the stimulus induced response (the subject shown in Fig. 2B is mc1, compare with the response magnitude to the stimulus given in Table 2). These percentages differed between subjects.

To analyze the magnitude and phase shifts across all subjects for stimulation and rest the further analyzes were performed in the frequency domain. The 1024-point PSDs were first averaged across all resting and stimulation periods in each subject. The grand average (Fig. 3) showed three major features. One peak around 0.03 s^{-1} (termed VLFO in analogy to references (Hu *et al.*, 1999; Kuo *et al.*, 1998)) a second peak around 0.1 s^{-1} (LFO) and a broad peak around 1 s^{-1} corresponding to the heart rate of the subjects. The expected peak at the assumed respiratory frequency around 0.2–0.3 s^{-1} (HFO) (Elwell *et al.*, 1996) was surprisingly small (data not shown, compare Figs. 5 and 6). The difference between stimulation and rest was statistically significant for [Cyt-ox] for the LFO and at 0.04 s^{-1} for [oxy-Hb]. The difference at 0.04 s^{-1} for [deoxy-Hb] did not reach statistical significance (one sided paired *t* test, 11 *df*, $P < 0.05$, for all comparisons). Controls across the whole spectrum and the heart beat range showed no effect of the stimulation thus rendering aliasing or an increase in noise improbable (see Fig. 4).

To answer the question whether the oscillations differ in phase between parameters we performed a coherence and a transfer function estimate in the same epochs (Fig. 5). The analysis disclosed a positive phase relation of ~1 rad (corresponding to 1.6 s at 0.1 s^{-1}) between oxy-Hb and deoxy-Hb. The comparison between oxy-Hb and Cyt-ox as well as between tot-Hb and Cyt-ox showed a shift of ~ −1.5 rad (corresponding to −2.4 s at 0.1 s^{-1}) for the resting condition and was statistically significantly increased to ~ −2 rad (corresponding to −3.9 s at 0.1 s^{-1}) by the stimulus. Since all NIRS parameters are derived from the changes in light attenuation across the NIR spectrum, artefacts are likely to mimic a high coherence at false frequencies. This may in part explain the much higher coherence across the whole spectrum when compared with the $COH^{aBP \rightarrow [chromophore]}$ as performed in experiment 2. Note, however, that the $COH^{[oxy\text{-}Hb] \rightarrow [deoxy\text{-}Hb]}$ shows clear peaks at the LFO and the HR frequencies while the $PHA^{[oxy\text{-}Hb] \rightarrow [deoxy\text{-}Hb]}$ is zero for the HR as opposed to the clear phase shift for the LFO frequencies. $COH^{[deoxy\text{-}Hb] \rightarrow [Cyt\text{-}ox]}$ and $PHA^{[deoxy\text{-}Hb] \rightarrow [Cyt\text{-}ox]}$ resulted in noisy spectra without clearly identifiable peaks (data not shown).

Experiment 2: Hypercapnia

Hypercapnia attenuated the LFO while enhancing the respiration induced HFO. This in fact was the only condition in which a clear peak was detected in the

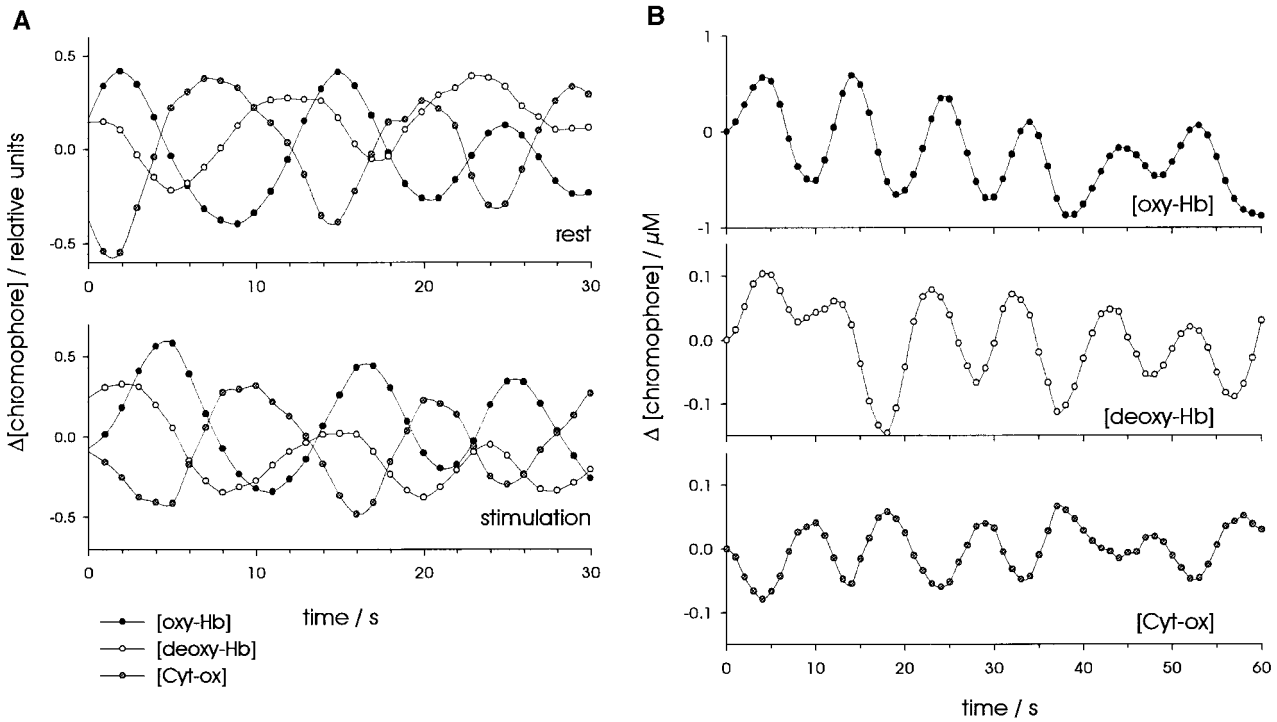


FIG. 2. Examples of unaveraged time-courses in two single subjects. (A) LFO in a single subject during stimulation and rest. To illustrate the phase shift between the oscillations in the three different parameters, the amplitude in all parameters was normalized (1 relative unit corresponds to the maximal amplitude within the epoch of 102.4 s, of which 30 s are shown). (B) provides an example from the resting period in another subject in micromoles. Note that the scaling for [oxy-Hb] is 50 times that of [deoxy-Hb] and [Cyt-ox].

range of the spontaneous respiratory frequency (Fig. 6). The differences between normo- and hypercapnia held true in all subjects for [deoxy-Hb] and in three subjects for [oxy-Hb]. The switch back to room air reduced the HFO back to baseline levels, while LFO power only partly recovered (data not shown). Differences between rest and visual stimulation were not analyzed, due to small sample size.

Experiment 3: Contribution of Skin Vasomotor Oscillations

There is a long standing discussion as to the contribution of extracerebral tissue to the NIRS parameter changes. Also, oscillations of the skin vasculature, at frequencies similar to those investigated here have been described (Kvernmo *et al.*, 1998). Therefore we tried to exclude the possibility that the oscillations described here are merely a phenomenon of skin blood flow oscillations. A simple way to simultaneously monitor NIRS parameters in different sampling volumes is to use different inter optode distances (IOD). With small IODs the sampling volume is dominated by the upper layers of the tissue, whereas only larger IODs will allow for a sampling from deeper layers. The principle used by commercial monitoring systems (Grubhofer *et al.*, 1999) is illustrated in Fig. 7a. It should be noted, though, that parameter changes in the upper layers will always contribute to the signal; however, the ratio of intra- to extracerebral contribution differs with IOD. (For a more detailed discussion and simulation based visualizations of this issue, which is beyond the focus of the present paper see Firbank *et al.*, (1998).)

The results (Fig. 7b) show that for [oxy-Hb] the slow oscillations detected with a 0.5 cm IOD are smaller compared to those using the IOD used in experiments 1 and 2, whereas for [deoxy-Hb], they are about the same size at the VLF but greater for the small IOD in the LFO region. When enhancing the power of the LFO by forced respiration at 0.1 s^{-1} (see Diehl *et al.* (1995)) the power of the LFO is typically increased 10-fold in [oxy-Hb] again with a predominance for the longer IOD.

While power does thus not allow for a clear differentiation between different sampling depths, the above described phase shift between [oxy-Hb] and [deoxy-Hb] was seen only for the long IOD (see Fig. 7c). This shift may therefore be characteristic for the lower layers, which are only sampled when using a longer IOD.

Coherence and Phase between aBP and NIRS Signals

In the subjects in whom aBP was monitored without major artefacts coherence and phase between the aBP and the three NIRS parameters was assessed. The

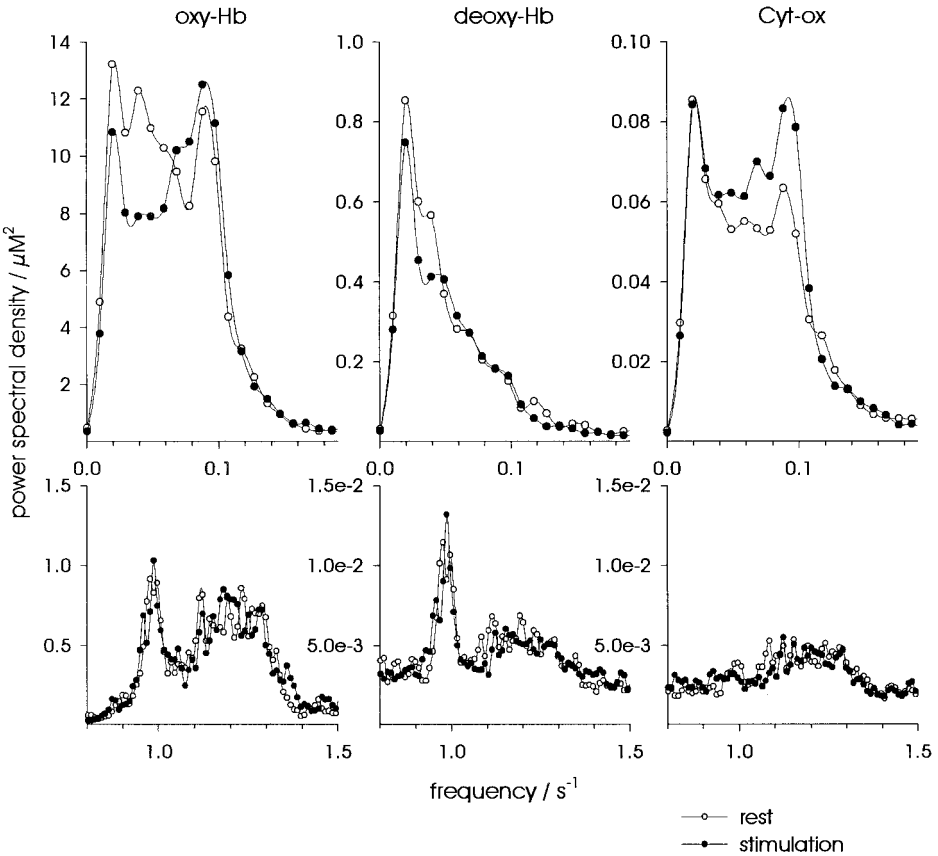


FIG. 3. PSD across all subjects and epochs (1024 data points, Hanning filter, no overlap) for stimulation (filled) and rest (open circles). The upper row shows the low frequency range below the corresponding features of the spectrum around the heart rate are shown. Note different scaling for the different parameters and the different scaling of the frequency range for the two rows of graphs. PSDs were performed in the data after conversion into μM.

results showed strong coherence in the heart-rate range (data not shown) and a weaker coherence in the range of the LFO (Fig. 8). Coherence was strongest between aBP and [oxy-Hb] phase shift between these parameters, however, was close to zero in the LFO range. The phase shift for the LFO between aBP and [deoxy-Hb] as well as aBP and [Cyt-ox] were of similar magnitude of those seen between [oxy-Hb] and the

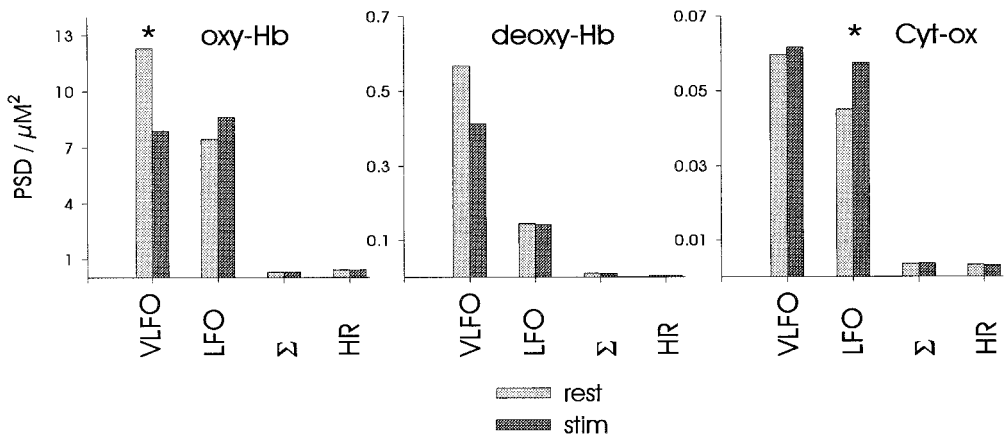


FIG. 4. PSD at 4 different spectral windows. Stimulation (dark columns) and rest (light columns) values were compared by *t* statistics across subjects (paired one-sided, 11 dof, $P < 0.05$ indicated by *). The following windows are shown (compare Fig. 3): VLFO = 0.04 s^{-1}; LFO = Σ(0.08–0.11 s^{-1}); HR = Σ(0.8–1.4 s^{-1}); Σ = Σ(0–5 s^{-1}). Note different scaling for the different parameters.

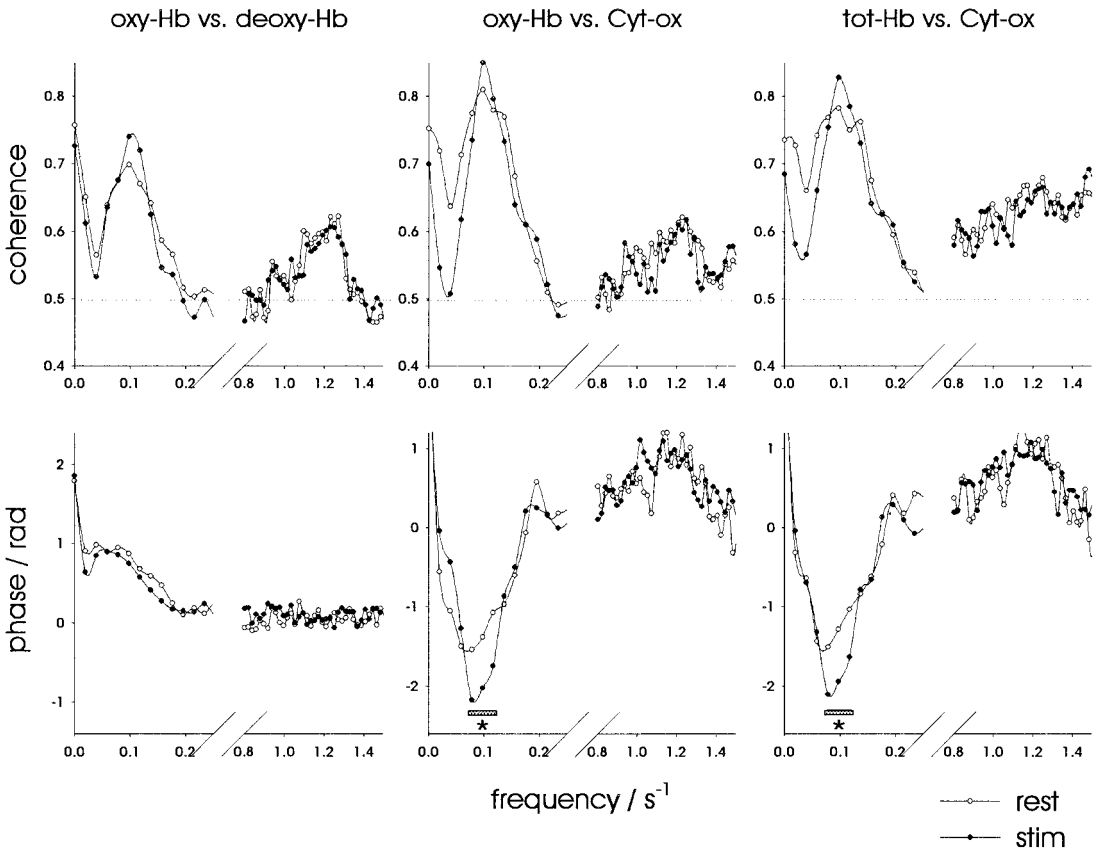


FIG. 5. COH and PHA spectra for the comparisons: [oxy-Hb] → [deoxy-Hb] (left), [oxy-Hb] → [Cyt-ox] (middle), [tot-Hb] → [Cyt-ox] (right). Open circles denote resting, closed circles denote stimulation epochs. 512 point coherence and transfer function estimates were performed in the 1024 point epochs, overlap was 256. Only the angle of the transfer function estimate was analyzed (PHA). Note that only when coherence is >0.5 values for PHA can be considered relevant.

latter two parameters as seen in experiment 1. Note that the positive phase shifts for [Cyt-ox] beyond 0.1 s^{-1} may be artefactual since coherence is lower than 0.5 (dashed line in Fig. 8).

DISCUSSION

The principal findings of this paper are: (1) Spontaneous low frequency oscillations in [oxy-Hb], [deoxy-Hb], and [Cyt-ox] can be detected noninvasively in the human cerebral cortex by NIRS. They are roughly of the same magnitude as the stimulus induced changes. (2) The oscillations below the respiratory frequency (HFO) are centered around 0.1 s^{-1} (LFO); a second peak in the frequency analysis centres around 0.04 s^{-1} (VLFO). (3) Both, LFO and VLFO are altered in power by functional activation of the cortical area investigated, the attenuation of VLFO power in [oxy-Hb] and the augmentation of LFO power in [Cyt-ox] reaching statistical significance. Hypercapnia results in an attenuation of the LFO in [oxy-Hb] and [deoxy-Hb]. (4) There is a phase shift between [oxy-Hb] and [deoxy-Hb] of about 1 rad (corresponding to ~ 1.6 s at this frequency) for the LFO, which is only detected when sampling from deeper layers (i.e., cerebral tissue) and is not present for the oscillations of similar frequency in predominantly skin and skull. The phase between [tot-Hb] and [Cyt-ox] for the LFO amounted to about −1.5 rad (~ −2.4 s at 0.1 s^{-1}) and was statistically significantly augmented by the stimulus.

The discussion of these findings is structured as follows: first we discuss potential problems of the NIRS methodology with respect to the sampled volume and the validity of the parameters. We then compare the findings to data from (a) animal studies, (b) fMRI studies in humans, and (c) TCD studies in human subjects and patients. The goal is to probe our hypothesis that the oscillations seen in the different NIRS parameters may help to define a measure of focal autoregulatory mechanisms of the cortical vasculature.

Validity of NIRS Recordings

Extra- versus intracerebral contribution. The validity of the findings critically depends on whether the changes monitored can be ascribed to truly cerebral

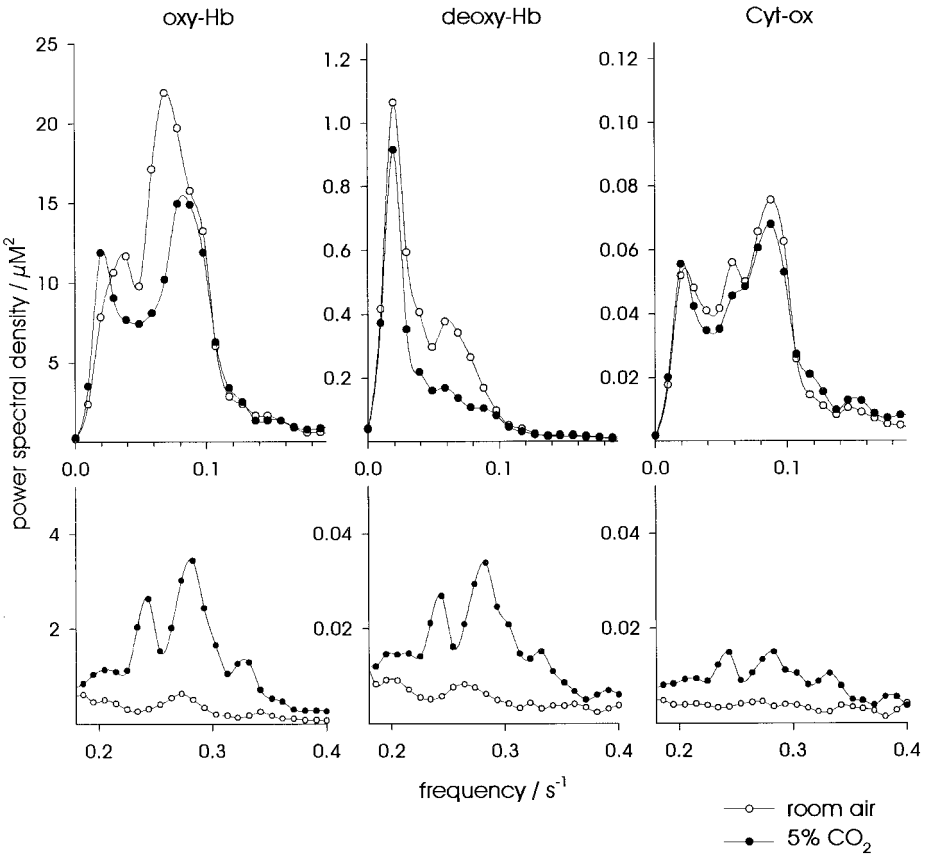
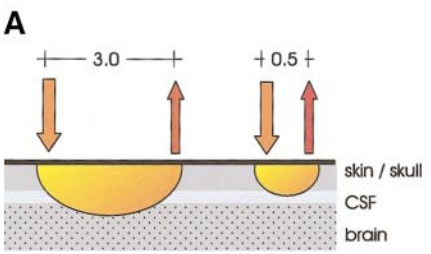
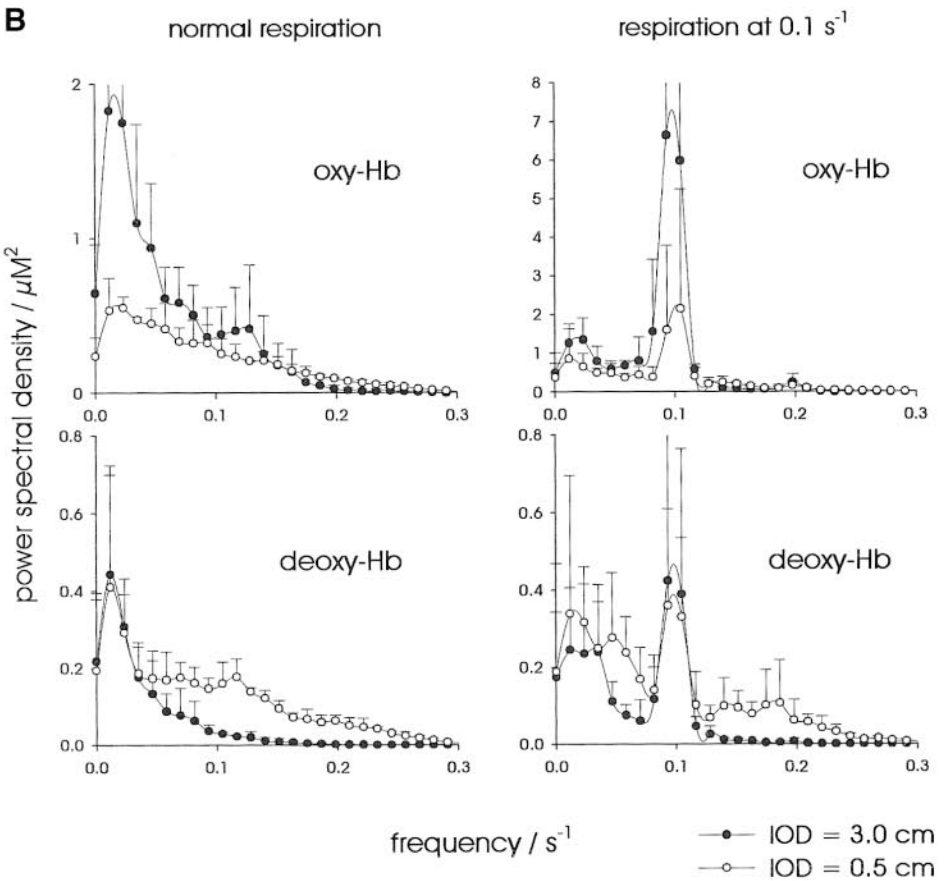


FIG. 6. Attenuation of the LFO by hypercapnia, mean across all subjects. The upper row shows that in [oxy-Hb] and even more clearly in [deoxy-Hb] the peak around 0.1 s^{-1} is attenuated by hypercapnia (closed circles). The respiration induced oscillations were enhanced during hypercapnia. In [deoxy-Hb] and [Cyt-ox] they barely produced any PSD feature during normocapnia (open circles). Switching back to normocapnia yielded baseline features for the HFO, the LFO power only recovered partially (data not shown). Note different scalings for the parameters.

hemodynamic and metabolic changes. The here applied NIRS technique, sampling through the intact skin and skull will also detect changes in skin oxygenation of similar frequencies due to changes in skin blood flow as have been described by laser doppler flowmetry in humans (Stauss *et al.*, 1999, 1998; Kvernmo *et al.*, 1998). In fact the results of our 3rd experiment show oscillations in [deoxy-Hb] and [oxy-Hb] also when using a probe separation of 0.5 cm, too small to sample from cerebral tissue. Using a probe separation of 3-cm theoretical considerations (Okada *et al.*, 1995) suggest that a considerable amount of the signal changes originates from hemodynamic changes in the pial vessels while predominant contribution of small (capillary?) vessels has also been postulated based on MC simulations (Liu *et al.*, 1995). Due to high interindividual variability of the local anatomy we consider reliance on these theoretical considerations insufficient. We argue

FIG. 7. (a) Sketch of the different sizes of volumes sampled by NIRS, when using different interoptode distances (IOD). With the small IOD absorption changes in the upper layers will dominate the signals detected. Only when using a longer IOD deeper tissue will substantially contribute to the changes monitored. (It should be noted that the colored structure denotes a grossly simplified sampling volume. The distribution of the photons in the tissue is better described by a "cloud" and extends into all directions from the light emitting probe. For a more detailed discussion and visualisation of the issue based on simulation data see Firbank *et al.* (1998). (b) PSDs of changes in [oxy-Hb] and [deoxy-Hb] sampled simultaneously with two different interoptode distances (IOD). Closed circles denote long IOD and open circles small IOD. Right hand graphs show the spectra during respiration at 0.1 s^{-1}. Since the IOD contributes to the magnitude of the concentration changes calculated by the modified Beer–Lambert law, the small IOD also enhances the noise of the measurement. Assuming that the nonbiological noise is of higher frequency the concentration changes were smoothed by a gliding average of 10 data points prior to the frequency domain analyzes. While power for the IOD of 0.5 cm was higher across all frequencies without this smoothing procedure (data not shown) this offset was successfully attenuated by the procedure (see Discussion). Scales differ for the upper two graphs. (c) COH and PHA between oxy-Hb → deoxy-Hb for the two IODs (compare Fig. 7b). Note the clear phase shift between oxy-Hb and deoxy-Hb being present only for the longer IOD.


A
3.0
0.5
skin / skull
CSF
brain


B
normal respiration
respiration at 0.1 s-1
oxy-Hb
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power spectral density / μM2
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IOD = 3.0 cm
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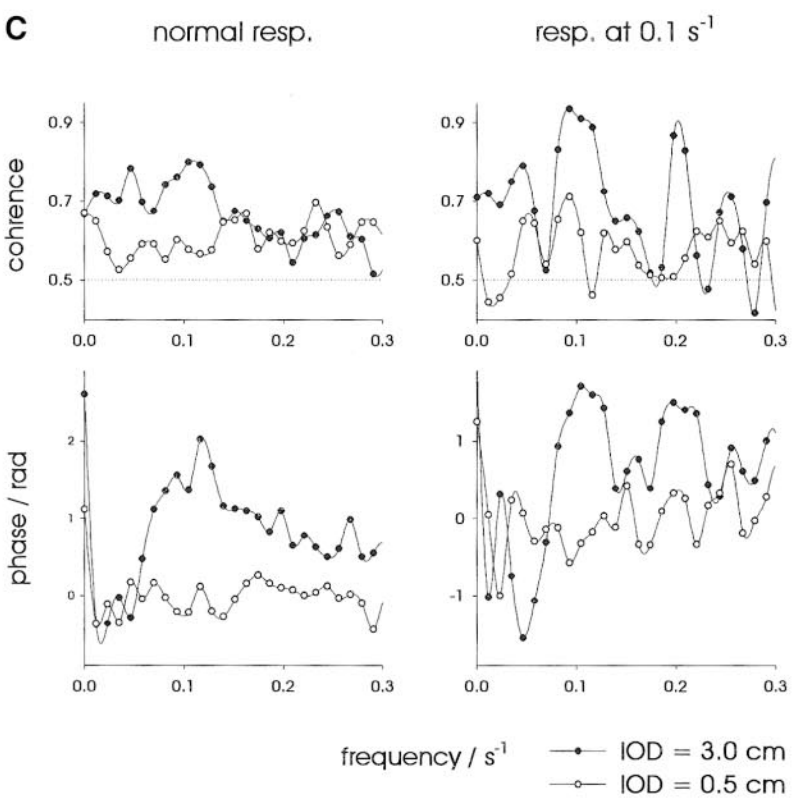
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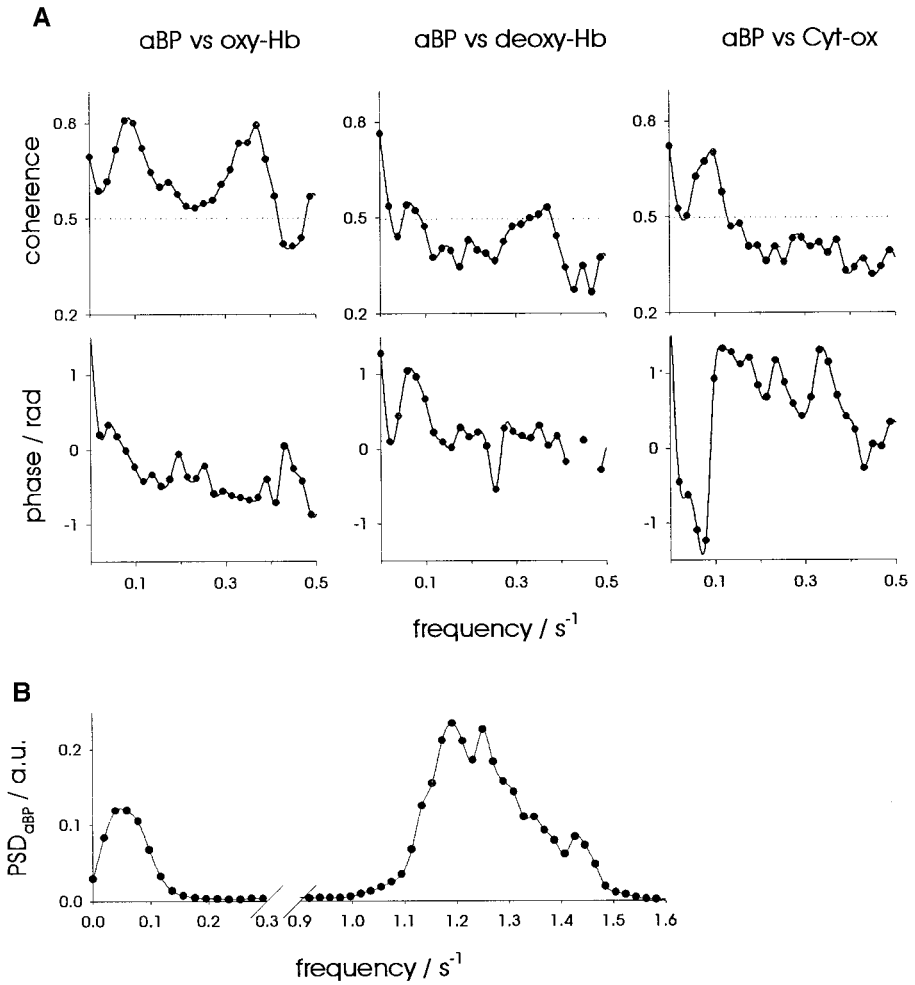

FIG. 8. (A) Phase and coherence relations between aBP and the three NIRS parameters, across all subjects (n = 3). The dashed line denotes a coherence of 0.5. Phase shifts corresponding to lower coherence values may be artefactual. (b) PSD of the aBP was measured in arbitrary units (a.u.) since changes rather than absolute values were assessed (see Methods and Analysis). The two most prominent features of the spectrum were around the LFO and the heart rate frequencies. Compare with Fig. 5.

that four findings in the present data support a substantial contribution of cerebral oxygenation changes to the signals measured: (1) The tissue illuminated showed a clear vascular and metabolic response to the visual stimulus, which is in line with our previous functional NIRS studies (Obrig and Villringer, 1997). (2) Power spectral density and phase shift between parameters were altered by the stimulation. (3) There is a phase shift between [oxy-Hb] and [deoxy-Hb] only seen when sampling with the longer probe separation. (4) If the oscillations were to solely stem from the skin, their magnitude should be smaller when sampling with a larger probe separation, due to a partial volume effect.

Cytochrome-oxidase. Another concern inherent to the here used technology is as to the significance of the changes in Cytochrome-oxidase redox state. This discussion mainly addresses the probability of a change in response to physiological stimuli, i.e., a fairly small change in oxygen demand. The response to the stimulus elicited in experiment 1 (see Fig. 1 and Table 2) shows remarkably stable recordings for the [Cyt-ox] parameter compared to the changes in the hemoglobins, making a simple cross-talk rather improbable. Also in a recent study (Heekeren *et al.*, 1999) we found that the changes in light attenuation induced by visual stimulation can be fitted with essentially randomly distributed residuals when assuming a contribution of Cytochrome-oxidase, while a two component fit with the extinction coefficients of oxy-Hb and deoxy-Hb alone will leave residuals similar in shape to that reported for the difference spectrum of Cytochrome-oxidase. It should be noted though that insufficient correction for changes in pathlength due to absorption changes may mimic a response. Mayhew and coworkers (Mayhew *et al.*, 1999) have shown that the initial deoxygenation in response to a stimulus, the initial "dip" (Malonek *et al.*, 1997) can be produced by insuf-

ficient pathlength correction. In the same publication a stimulus induced increase in [Cyt-ox] oxidation is not found when the "V-signal," i.e., the LFO, is separated from the stimulus response. We argue, that (1) spectroscopy in the visible spectrum predominantly monitors the heme a/a3 center as opposed to the CuA center of the enzyme monitored in the near-infrared; (2) the spectral region in the near-infrared is less susceptible to DPF misjudgements than the visible spectrum used in the animal study, also the LFO are clearly superimposed on the Cyt-ox response; (3) our previous study suggests a change in Cyt-ox redox-state in response to a visual stimulus; (4) the phase shift between the hemoglobins and Cyt-ox, moreover its alteration by the stimulation renders a simple cross talk improbable.

Physiological Relevance of the Findings

Invasive/animal studies. One of the most striking findings in the present study is the phase shift between the vascular and the metabolic parameters. A number of invasive studies in different species report similar phase shifts. Dora and Korvach (1981) describe three different types of oscillations in the cat cerebral cortex. Those occurring spontaneously at a frequency of 0.08–0.16 s^{-1} exhibit a phase shift of 2–4 s between the vascular volume and the NAD/NADH redox state, suggesting the vascular changes to be primary. Vern and coworkers, however, conclude the contrary reporting on cytochrome oxidase oxidation (CYT) lagging 2–3 s behind blood volume (CBV) oscillations as measured by dual wavelength spectrophotometry in the rabbit (Vern *et al.*, 1997) and the cat (Vern *et al.*, 1988). Their conclusion is prompted by the observation of metabolic changes in the absence of blood volume changes, a finding not shared by other investigators. Mayevsky and Ziv (1991) find a phase difference between blood volume and NADH redox state in a rat stroke model, the changes in phase in response to the ischemia are not analyzed. Concerning the influence of stimulation in a study by Mayhew *et al.* (1999) investigating oxygenation and metabolic changes in the rat whisker barrel cortex, a 1-s lag between the oxy-Hb and the Cyt-ox signal is seen for the spontaneous 0.1 s^{-1} oscillations. In contrast to our findings there is no evidence of a phase shift in response to the stimulus. The stimulation protocol, however, applies short stimuli of 1 s duration only, also a Cytochrome-oxidase response is not elicited or is being buried in the rather large spontaneous oscillations.

One of the primary issues of the present paper, the "modulatability" of the oscillations by functional cortical activation invokes the question as to the oscillations' origin. Neuronal, metabolic, and vascular (Hudetz *et al.*, 1998) origin have been postulated but in part similar findings have been interpreted in contrary direction (see above Vern *et al.* (1988) versus Dora and Korvach (Dora and Kovach, 1981)). Golanov *et al.* (1994) report oscillations of 0.1 s^{-1} being preceded by EcOG bursts of a specific type, and demonstrate that these cerebrovascular waves can be evoked by stimulation of cerebellar or medullar sites (Golanov and Reis, 1996). In the discussion of their findings, however, they state these waves to be different from the vasomotion, due to shape, spontaneity, and response to aBP changes.

To sum up, the literature provides broad evidence of the existence of both metabolic and vascular oscillations in different species. If vascular and metabolic oscillations are measured simultaneously they differ in phase. To our knowledge as yet the influence of sustained physiological activation on these oscillations has not been studied.

BOLD contrast fMRI studies. In BOLD contrast fMRI studies the oscillations have first been recognized as a source of physiological noise (Mitra *et al.*, 1997; Biswal *et al.*, 1996) unrelated to heart rate and respiration. Similarly a recent NIRS study has pointed out the relevance of these oscillations for functional imaging studies (Elwell *et al.*, 1999). Biswal and coworkers (Biswal *et al.*, 1995) were the first to report on connectivity maps based on low frequency oscillations. This technique has been used to impressively demonstrate functional systems in resting state series of images. Decreases in the so defined functional connectivity have been tested by pharmacological intervention (Li *et al.*, 2000) and hypercapnia (Biswal *et al.*, 1997). However, a number of questions remains unresolved. First, coherence of the assumed underlying spontaneous LFO has been demonstrated to be very localized. Vern *et al.* report an in-phase relation of homologous areas of 50 mm^2, which might reach the size of at the most a couple of pixels but does not explain the coherence of rather large cortical areas. Other authors report even smaller areas of synchronicity in the rat (Mayhew *et al.*, 1996; Mayevsky and Ziv, 1991) or show coherence of capillary flow only after the application of a NOS-inhibitor (Biswal and Hudetz, 1996; Kleinfeld *et al.*, 1998; Dirnagl *et al.*, 1993). In the human an invasive study showed asynchronous behaviour of oxygen tension and blood flow oscillations also in the range of millimetres (Cooper *et al.*, 1966). In the present study we detect VLFO and LFO even though sampling volume will be rather on the order of a centimeter, confirming that even in larger areas the oscillations do not completely cancel out due to phase shifts. Second, the frequency range analyzed in the fMRI connectivity studies, i.e., low frequency below 0.08 s^{-1}, is not in agreement with the typical range reported in the literature. With respect to the present findings two explanations are possible: (i) the VLFO rather than the LFO are the basis of the connectivity maps, (ii) BOLD contrast is generated by a complex interplay of oxygen

uptake, blood volume, and flow velocity, which may alter its spectral profile. Both issues are connected, since it may be helpful to analyze different parameters and frequency ranges as for their aptitude to disclose connectivity. A combined approach of NIRS and fMRI (Kleinschmidt *et al.*, 1996) may help to clarify the issue.

The effect of attenuation of the LFO by hypercapnia is consistent between the LDF findings (Hudetz *et al.*, 1995) in the rat, fMRI results in the human (Biswal *et al.*, 1997) and the presently reported data. The fMRI study also reports an increase in HFO, i.e., frequencies related to the respiratory cycle, which is in line with our findings, though it should be noted that the frequency reported by the authors for the LFO and HFO is surprisingly low (about 0.01 and ~0.13 s^{-1}, respectively, according to the figure, which is unusual for spontaneous respiration).

Transcranial Doppler sonography (TCD) studies in the human. The close connection between autoregulation and the here described spontaneous oscillations is agreed on by a number of investigators (Hudetz *et al.*, 1998). For example, lowering the aBP has been shown to result in a magnitude increase of the oscillations (Hudetz *et al.*, 1992) down to the lower level of the autoregulation resulting in a U-shaped dependence (Morita-Tsuzuki *et al.*, 1992). More directly and with an extension into clinical application this has been addressed by TCD recordings in the human.

The method extensively studied by a number of groups (Giller and Iacopino, 1997; Panerai *et al.*, 1998; Blaber *et al.*, 1997; Diehl *et al.*, 1995) uses the spontaneous oscillations in aBP to assess the cerebrovascular reactivity. Zhang *et al.* (1998a, 1998b) introduced transfer function analysis to analyze gain and phase of the LFO and VLFO between aBP and TCD recordings. Their TCD spectra (compare Fig. 1 in Zhang *et al.*, 1998a) exhibit the very peaks found in the present study, even showing the loss of the HF peak due to inter-subject respiratory variability. Interestingly the phase relationship between aBP and TCD velocity changes resembles that found between [oxy-Hb] and [deoxy-Hb], the phase reaching about 1 rad (~1.6 s) at 0.1 s^{-1} for both comparisons. Also beyond 0.2 s^{-1} the phase shifts reach zero, indicating that the cerebrovascular autoregulation can be conceived as a high pass filter. We interpret this striking similarity as follows: the autoregulatory effect seen in the TCD recordings is produced as a net-effect of all arterioles in the MCA territory. On the other hand NIRS will sample from a more localized mixture of the capillary, arteriolar, and venous compartment. A decrease in [deoxy-Hb] can be explained only by an increase in blood flow velocity, if oxygen consumption is unaltered. [Oxy-Hb] on the contrary can be altered by both volume and flow variations. The thus hypothesized difference between the oscillations in blood volume and flow velocity may well reflect *focal* autoregulation as opposed to the TCD measurements monitoring autoregulation in the whole territory of the large vessel insonated. The hypothesis is supported by (1) the finding that $PHA^{aBP \rightarrow [oxy\text{-}Hb]}$ is zero, whereas $PHA^{aBP \rightarrow [deoxy\text{-}Hb]}$ amounts to 1 rad at 0.1 s^{-1} (see Fig. 8); (2) the heart beat induced pulsatile changes in pressure are much more pronounced in [oxy-Hb], suggesting that the systemic changes will be more clearly seen in [oxy-Hb]; (3) differences between volume and flow effects have been described in fMRI in response to functional stimulation (Mandeville *et al.*, 1999). Similar phase shifts between aBP and MCA flow velocity are reported by Blaber *et al.* (1997) (−1 rad, due to inverse comparison), Hu *et al.* (1999) (0.8 rad/~1.3 s at 0.1 s^{-1}), and Diehl *et al.* (1995) (1.2 rad/~1.9 s at 0.1 s^{-1}).

The applicability of the phase shift between aBP and MCA flow velocity as an indicator of disturbed autoregulation in carotid stenosis (Diehl *et al.*, 1991) and small vessel disease (Bazner *et al.*, 1995) has been demonstrated. We currently investigate focal changes in the oscillations' amplitude and phase in stroke patients.

Coherence and phase comparisons between NIRS parameters. As a caveat it should be noted that the comparison between two parameters, e.g., oxy-Hb and deoxy-Hb, calculated from the same basic parameter at different wavelengths, i.e., the changes in light intensity (in units of optical densities) in the illuminated tissue, may bear some problems. Any noise originating from movement artefacts will mimic coherence between the derived parameters, since it influences the changes in light attenuation across the whole optical spectrum. As seen when comparing Figs. 5 and 8 the coherence between two NIRS parameters is greater than that between aBP as measured by the finger plethysmography and one of the NIRS parameters. Besides the greater number of subjects for the within NIRS comparison coherent noise may be a reason for this difference. However the frequency dependence of the coherence and phase shifts evaluated between NIRS parameters is in line with the reported high pass filter characteristics postulated for the cerebral vasculature. Note that at the frequency of the heart beat around 1 s^{-1}, we find a strong coherence without any phase shift between oxy-Hb and deoxy-Hb. For the comparison between oxy-Hb, tot-Hb, and Cyt-ox, the fact that stimulation alters the phase shift in a statistically significant manner makes non-physiological noise a bad candidate for the coherence and phase shifts reported.

CONCLUSIONS

The present study is the first to investigate slow spontaneous oscillations in vascular and metabolic parameters in the adult human brain under the influence

of stimulation and hypercapnia. For the resting brain Elwell and coworkers have previously reported similar results as to magnitude and frequency of vasomotion related oscillations in the visual cortex (Elwell *et al.*, 1999), while Eke *et al.* describe a fractal analysis model to describe the oscillations in NIRS parameters (Eke and Herman, 1999). The present finding of an alteration in power by hypercapnia and physiological stimulation encourages the investigation of their alteration in cerebrovascularly diseased patients. They may be altered by impaired autoregulation. Their magnitude of about the size of the stimulus induced changes renders them a potential nuisance to functional activation studies especially when using single trial set-ups. The phase shift of vascular-metabolic parameters as seen by a sustained visual stimulus may further the understanding of the coupling of brain activity to the vascular response.

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