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Cerebral haemoglobin oxygenation during sustained visual stimulation—a near-infrared spectroscopy study

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SUMMARY

Using near-infrared spectroscopy, we investigated the time-course of the concentrations of oxygenated haemoglobin, [oxy-Hb], and deoxygenated haemoglobin, [deoxy-Hb], in the occipital cortex of healthy human adults during sustained visual stimulation. Within a few seconds after stimulation (by coloured dodecahedron), we observed a decrease in [deoxy-Hb], peaking after 13 s ('initial undershoot'). In the subsequent 1–2 min, in seven out of ten subjects, [deoxy-Hb] gradually returned to a plateau closer to the baseline level. After cessation of stimulation, there was a 'post-stimulus overshoot' in [deoxy-Hb]. There was a statistically significant correlation between the size of the 'initial undershoot' and the 'post-stimulus overshoot'.

The concentration of oxyhaemoglobin increased upon functional activation. However, in the mean across all subjects there was no 'initial overshoot'. After approximately 19 s it reached a plateau and remained constantly elevated throughout the activation period. After cessation of activation there was a 'post-stimulus undershoot' of oxyhaemoglobin.

It is important to consider the time-course of haemoglobin oxygenation when interpreting functional activation data, especially those data obtained with oxygenation-sensitive methods, such as BOLD-contrast fMRI.

1. INTRODUCTION

Brain activity is associated with an increase in cerebral blood flow (Roy & Sherrington 1890; Fox *et al*. 1986; Olesen 1971; Villringer & Dirnagl 1995). It has been shown by several different methods (Fox & Raichle 1986; Ueki *et al*. 1988) that this increase in blood flow is not matched by a proportional increase in oxygen consumption. In agreement with this mismatch, it has been shown by optical methods, fMRI and O_2-electrodes, that within a few seconds after the onset of elevated brain activity cerebral haemoglobin oxygenation rises (Cooper *et al*. 1975; Bandettini *et al*. 1992; Frahm *et al*. 1992; Kwong *et al*. 1992; Ogawa *et al*. 1992; Villringer *et al*. 1993).

Recently, a number of fMRI studies employing the [deoxy-Hb]-related BOLD-contrast have addressed the time-course of this hyperoxygenation during prolonged stimulation. There is general agreement, that during motor activation tasks the elevated BOLD signal (presumably corresponding to a drop in [deoxy-Hb]) persists throughout longer activation periods of several minutes. However, there is controversy whether this is also true for prolonged *visual* stimulation. Some authors have reported that the BOLD signal increase gradually returns to baseline or near-baseline values (Hathout *et al*. 1994; Frahm *et al*. 1996), whereas other authors have reported a constant elevation of the deoxyhaemoglobin-related fMRI signal (Bandettini *et al*. 1995).

One possible reason for this discrepancy may be differences in fMRI methodology, since fMRI measures deoxyhaemoglobin only indirectly. In this study we therefore used an MRI-independent approach to address this issue.

Near-infrared spectroscopy (NIRS) is an optical method (see Chance (1991) for a review) which non-invasively measures changes in cerebral [deoxy-Hb] and [oxy-Hb] in human adults (Elwell *et al*. 1992). It has recently been shown by several groups that NIRS permits the assessment of changes in cerebral haemoglobin oxygenation during various types of functional activation, such as cognitive activation (Hoshi & Tamura 1993; Villringer *et al*. 1993), visual activation (Kato *et al*. 1993; Villringer *et al*. 1993; Meek *et al*. 1995; Wenzel *et al*. 1996) and motor activation (Obrig *et al*. 1996).

In this study, we used this approach to assess changes in haemoglobin oxygenation in the occipital cortex during sustained visual activation. (Preliminary data have been presented previously in abstract form (Heekeren *et al*. 1996).)

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2. MATERIALS AND METHODS

(*a*) *Near-infrared Spectroscopy* (*NIRS*)

The technique of NIRS relies on the application of a modified Lambert–Beer law to measured variations in attenuation, in order to determine the corresponding changes in the concentration of tissue chromophores. The method has been described in detail elsewhere (Cope & Delpy 1988). Briefly, attenuation of light by tissue depends on scatter and absorption. In a highly scattering medium, such as brain tissue, optical attenuation can be expressed as follows:

$$\text{attenuation} = \alpha c d B + G,$$

where α is the specific extinction coefficient of the absorbing compound measured in $\mu\text{molar}^{-1}\,\text{cm}^{-1}$, c is the concentration of the absorbing compound measured in μM concentrations, and d is the interoptode spacing measured in cm. B, the differential path length factor (DPF) (Delpy *et al*. 1988), accounts for the increased optical path length due to scattering, and the additive term, G, for scattering losses. Under the assumption that d, B, and G remain constant during the measurement period, concentration changes can be calculated as follows:

$$\Delta c = (\text{change in attenuation})/\alpha d B.$$

Changes in concentration of a number of chromophores can simultaneously be computed from the changes in attenuation at a number of wavelengths using an algorithm incorporating the relevant extinction coefficients for each wavelength and chromophore (Wray *et al*. 1988). The DPF for the adult head has been measured by time-of-flight methods (Essenpreis *et al*. 1993; Van der Zee *et al*. 1992) and in the frequency domain by phase-shift measurements (Duncan *et al*. 1995). Duncan *et al*. (1995) investigated 100 subjects and reported a mean value of 6.26 (s.d. = 0.88) at 807 nm without gender difference. Since in the present study we did not measure DPF individually, for the determination of concentration changes we used this DPF. Assuming this DPF value of 6.26, the reported concentration changes in arbitrary units (a.u.) correspond to μM concentration changes.

(*b*) *NIRO-500*

We used a NIRO-500 system (Hamamatsu Photonics KK, Japan) to continuously measure changes in [oxy-Hb] and [deoxy-Hb] through the intact skull in reflection mode (Ferrari *et al*. 1986; Elwell *et al*. 1992). Light emitted from four laser diodes (wavelengths of 775, 825, 850, and 904 nm) was carried to the subject's head via an optical fibre (optode). Light reflected from the tissue was guided to a photomultiplier tube via a second optode. Data were acquired with a temporal resolution of 1 s, and changes in optical densities were converted to changes in chromophore concentration according to the algorithm implemented in the near-infrared spectrometer (Elwell 1995).

(*c*) *Subjects*

Twelve healthy, right-handed adults were examined (six women and six men, mean age 29 years). Each subject gave informed consent to participate in the study.

(*d*) *Optode localization*

The calcarine sulcus varies strongly in relation to cranial landmarks (Steinmetz *et al*. 1989). Therefore, the optodes were horizontally positioned over the right occipital region at the level of the calcarine sulcus according to 3-D-reconstructed high-resolution MRI. The light-emitting optode was placed 1 cm to the right of the midline to avoid the sagittal sinus, and the light-collecting optode was placed 3.5 cm laterally to the first.

(*e*) *Stimulation protocol*

All measurements were performed while subjects were lying in a dark, quiet room. After dark adaptation, the first stimulus was presented as soon as stable baselines for [oxy-Hb] and [deoxy-Hb] were reached.

The visual stimulus consisted of a multicoloured dodecahedron which was displayed on a computer monitor placed 2 m from the subject's head at eye level. Five minutes of stimulation were alternated with the presentation of a dark screen for three minutes. Each of the subjects complemented six to eight consecutive cycles.

(*f*) *Data analysis*

Data were related to an arbitrary zero calculated from the 6 s prior to the onset of stimulation, and averaged over all respective cycles. For statistical analysis we compared the following temporal windows.

(*i*) *Assessing the presence of an NIRS response in single subjects*

We compared two time frames of 6 s: the mean of the last 6 s prior to the onset of stimulation, and the mean of 6 s during the presumed peak response (mean 13–18 s after the onset of the stimulation) according to the results of our previous study (Wenzel *et al*. 1996). Only if there was a statistically significant response of [deoxy-Hb] during this time period did we include the subjects for further analysis.

(*ii*) *Assessing whether the drop in [deoxy-Hb] is constant over time during prolonged stimulation*

We averaged the data over 30 s (see figure 2) and compared the average of the frame preceding the stimulation (R), the initial response (average of [deoxy-Hb], frame A_1), the subsequent 30 s of stimulation (A_2) and the 30 s before the end of the stimulation period (A_{10}) by performing multiple paired t-tests (Bonferroni-adjusted).

(iii) Assessing whether there is a 'post-stimulus overshoot' of [deoxy-Hb]

We compared the mean [deoxy-Hb] of frames P_1/P_2 ($P_{1/2}$) and the mean of frames P_3/P_4 ($P_{3/4}$) (paired *t*-test).

A difference in [deoxy-Hb] between $A_{2\text{–}10}$ and A_1 may be called an 'initial undershoot' and a difference between $P_{1/2}$ and $P_{3/4}$ may be called a 'post-stimulus overshoot' of [deoxy-Hb]. Using bivariate correlation analysis we examined whether the magnitude of the 'initial undershoot' and the 'post-stimulus overshoot' are correlated.

3. RESULTS

Table 1 displays concentration changes for each subject during the period of the presumed peak versus the rest period (according to Wenzel *et al*. 1996). Nine of the twelve subjects showed an increase in [oxy-Hb] during this period which was statistically significant in five cases. Eleven out of 12 subjects showed a decrease in [deoxy-Hb], which was statistically significant in ten of 12 subjects. Only these ten subjects were included for further analysis.

In the included ten subjects, the mean concentration change during the presumable peak response was 0.23 ± 0.10 (mean $\pm$ standard error of the mean (s.e.m.)) arbitrary units (a.u.) for [oxy-Hb] and -0.21 ± 0.03 a.u. for [deoxy-Hb]. These changes were highly significant for [deoxy-Hb] ($p < 0.001$, paired *t*-test with 9 degrees of freedom (d.f.), two-tailed), and also statistically significant for [oxy-Hb] ($p < 0.05$, paired *t*-test with 9 d.f., two-tailed).

Table 1. *Mean differences in* [*oxy-Hb*] *and* [*deoxy-Hb*] ($\pm$*s.d.*, * $\pm$*s.e.m.*) *between the* 6 s *prior to stimulation onset and* 13–18 s *and level of significance* (*paired t-test*)

Subject	Δ[oxy-Hb] ± s.d. (*sig.*)	Δ[deoxy-Hb] ± s.d. (*sig.*)
#1	-0.16 ± 0.74 ($p = 0.590$)	-0.10 ± 0.09 ($p = 0.025$)
#2	0.42 ± 0.36 ($p = 0.011$)	-0.18 ± 0.04 ($p = 0.000$)
#3	0.07 ± 0.49 ($p = 0.691$)	-0.19 ± 0.22 ($p = 0.039$)
#4	-0.14 ± 0.30 ($p = 0.237$)	-0.18 ± 0.06 ($p = 0.000$)
#5	0.04 ± 0.24 ($p = 0.623$)	-0.21 ± 0.16 ($p = 0.006$)
#6	0.54 ± 0.34 ($p = 0.012$)	-0.33 ± 0.16 ($p = 0.004$)
#7	0.30 ± 0.23 ($p = 0.008$)	-0.19 ± 0.14 ($p = 0.006$)
#8	0.27 ± 1.02 ($p = 0.480$)	-0.15 ± 0.12 ($p = 0.010$)
#9	0.31 ± 0.95 ($p = 0.015$)	-0.15 ± 0.11 ($p = 0.004$)
#10	0.73 ± 0.41 ($p = 0.001$)	-0.42 ± 0.14 ($p = 0.004$)
grand average (##1–10)	0.23 ± 0.10* ($p = 0.028$)	-0.21 ± 0.03* ($p = 0.000$)
#11	0.19 ± 0.93 ($p = 0.585$)	0.04 ± 0.26 ($p = 0.665$)
#12	-0.18 ± 0.40 ($p = 0.318$)	-0.05 ± 0.12 ($p = 0.314$)

The grand average over these ten subjects is given in figure 1.

[Oxy-Hb] rises to its maximum during the first 19 s of stimulus presentation and remains almost stable during the entire 5 min of stimulation, after the end of the stimulation there is a post-stimulus undershoot which peaks after 16 s. Baseline values are reached approximately 15 s later.

The [deoxy-Hb] time-course is different. There is an initial decrease, which reaches its minimum about 13 s after the onset of the stimulation. Within the subsequent 10 s, [deoxy-Hb] starts to increase again and reaches a new level within 40 s after the onset of the stimulation. Whereas in some subjects [deoxy-Hb] returns to baseline or near-baseline values, the grand average seems to indicate a second plateau of [deoxy-Hb].

After cessation of the stimulation [deoxy-Hb] increases, and exceeds baseline values (post-stimulus overshoot) reaching a maximum approximately 16 s later. The concentration of deoxy-haemoglobin remains above baseline values for about 40 s, during which it gradually returns, reaching baseline values about 50 s after the end of the stimulation.

For figure 2, data were averaged over 30 s for each subject and the mean value of the last 30 s before stimulation onset (R) was set to 0.0 a.u. The individual measures were then averaged over all subjects.

To examine the time-course of [deoxy-Hb], we performed multiple paired *t*-tests (Bonferroni-adjusted) and compared R, A_1, A_2 and A_{10} (see table 2). The results, for [deoxy-Hb], show the following ($\alpha = 0.004$ due to the Bonferroni adjustment): [deoxy-Hb] (i) decreases during the first 30 s of activation ($R > A_1$, $p < 0.001$), (ii) increases again during the subsequent 30 seconds ($A_1 < A_2$, $p = 0.001$) and remains elevated ($A_1 < A_{10}$, $p = 0.004$), and (iii) does not significantly increase further (no significant difference between A_{10} and A_2).

Figure 3 displays the individual time-courses of [deoxy-Hb] for three subjects.

Subject #1 shows the same features as the grand average. There is an initial decrease in [deoxy-Hb] ($A_1 = -0.262$ a.u.), that is transient ($A_{2\text{–}10} - A_1 = 0.159$ a.u.). Subsequently, [deoxy-Hb] increases and reaches a plateau ($A_{2\text{–}10} = -0.1$ a.u.). After cessation of the stimulation there is a post-stimulus overshoot ($P_{1/2} - P_{3/4} = 0.064$ a.u.).

Subject #2 shows a transient decrease in [deoxy-Hb] ($A_1 = -0.111$ a.u., $A_{2\text{–}10} - A_1 = 0.09$ a.u.), too, but in this case [deoxy-Hb] reaches almost baseline values before the end of the stimulation ($A_{2\text{–}10} = -0.021$). There is also a post-stimulus overshoot ($P_{1/2} - P_{3/4} = 0.072$ a.u.).

Subject #3 shows a different time-course: there is an initial decrease of [deoxy-Hb] ($A_1 = -0.287$ a.u.), but in this case the subsequent increase in [deoxy-Hb] is very small ($A_{2\text{–}10} = -0.260$ a.u., $A_{2\text{–}10} - A_1 = 0.027$ a.u,). There is no post-stimulus overshoot ($P_{1/2} - P_{3/4} = -0.018$ a.u).

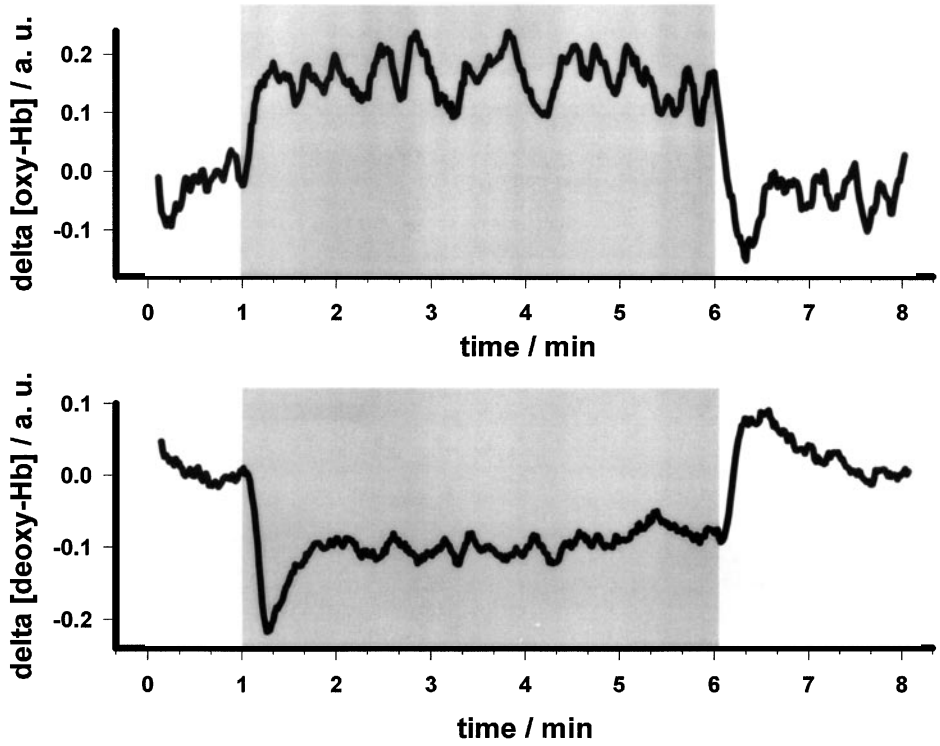

Figure 1. Grand average over ten subjects. The grey-shaded area depicts the 5 min of visual stimulation. Changes in [oxy-Hb] and [deoxy-Hb] are given in arbitrary units, which correspond to μM concentration changes when assuming a DPF of 6.26.

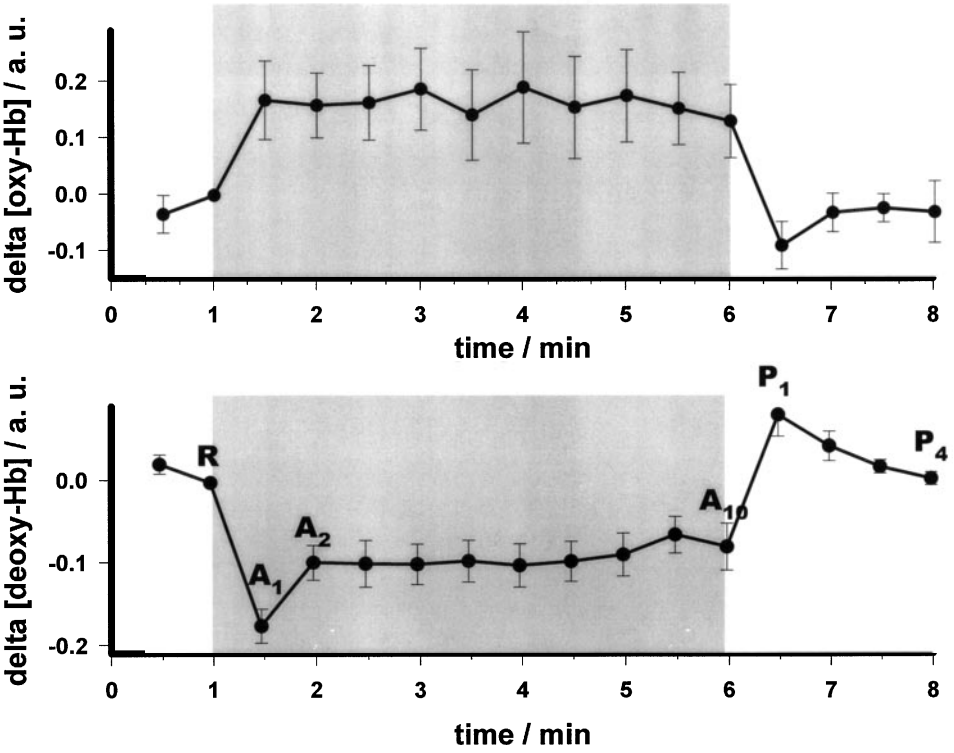

Figure 2. Grand average over ten subjects. The data are averaged over 30 s, error bars represent the s.e.m. Names of the frames are given (R, A_1, A_2,... A_{10}, P_1,... P_4). The grey-shaded area marks the 5 min of visual stimulation. Changes in [oxy-Hb] and [deoxy-Hb] are given in arbitrary units, which correspond to μM concentration changes when assuming a DPF of 6.26.

Table 2. *Multiple paired t-tests, comparing frames R, A_1, A_2, A_{10} (see figure 2). Mean paired differences in [deoxy-Hb] ($\pm$ s.e.m.)*

(Note $\alpha = 0.004$ because of the Bonferroni adjustment. Statistically significant results are in bold type.)

mean (**frame**) $\pm$s.e.m./a.u. (*sig.*)	*R*	A_1	A_2	A_{10}
R	—	**−0.174±0.02** (**$p < 0.001$**)	**−0.097±0.021** (**$p = 0.001$**)	−0.078±0.029 ($p = 0.024$)
A_1	**−0.174±0.02** (**$p < 0.001$**)	—	**0.077±0.049** (**$p = 0.001$**)	**0.096±0.025** (**$p = 0.004$**)
A_2	**−0.097±0.021** (**$p = 0.001$**)	**0.077±0.049** (**$p = 0.001$**)	—	0.020±0.015 ($p = 0.211$)
A_{10}	−0.078±0.029 (**$p = 0.024$**)	**0.096±0.025** (**$p = 0.004$**)	0.020±0.015 ($p = 0.211$)	—

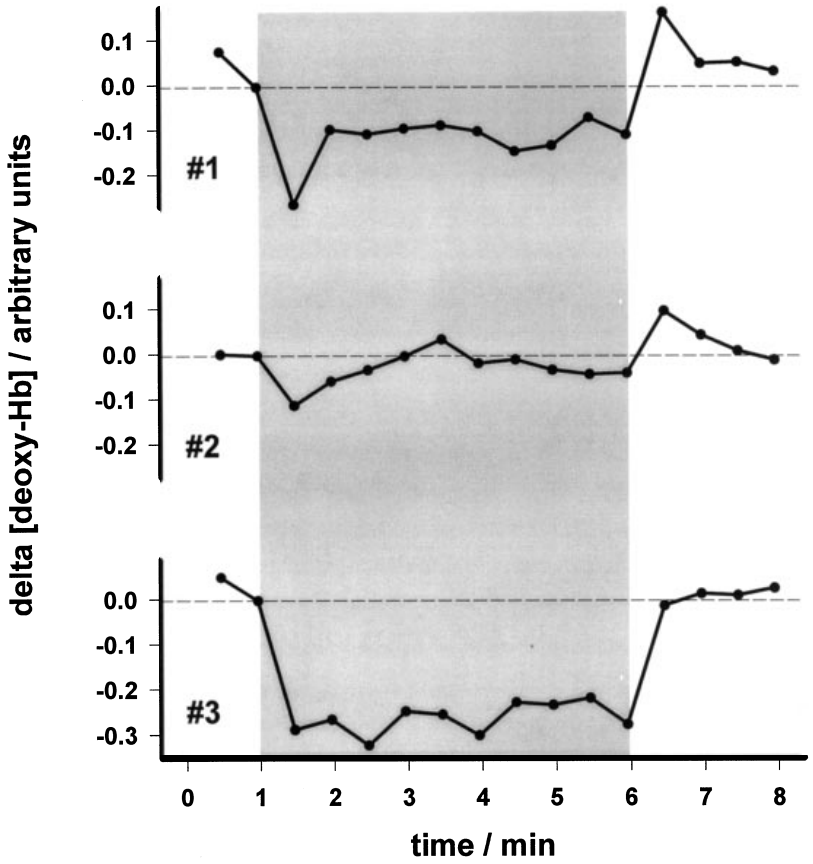

Figure 3. Individual averages of the time course of [deoxy-Hb] for three subjects that showed a statistically significant response in [deoxy-Hb]. Data are averaged over 30 s. The grey-shaded area marks the 5 min of visual stimulation. Changes are given in arbitrary units, which correspond to μM concentration changes when assuming a DPF of 6.26. Note that the range of the *y*-axis is the same for all subjects (0.44 a.u.).

$A_{2\text{-}10} - A_1$ was bigger than 0.0 in all subjects, a clear initial undershoot ($A_{2\text{-}10} - A_1 = 0.05$ a.u.) was seen in seven subjects (see table 3). $P_{1/2} - P_{3/4}$was bigger than 0.0 in seven subjects (see table 3).

The difference in [deoxy-Hb] between $A_{2\text{-}10}$ and A_1 (see figure 2) may be called an initial undershoot and a difference between $P_{1/2}$ and $P_{3/4}$ (see figure 2) may be called a post-stimulus overshoot of [deoxy-Hb]. The mean initial undershoot (mean $(A_{2\text{-}10})$ − mean (A_1)) of [deoxy-Hb] was 0.083±0.017 a.u. The presence of this undershoot was shown to be statistically significant ($p = 0.001$, one-sample *t*-test, 9 d.f., see table 3).

The mean post-stimulus overshoot (mean $(P_{1/2})$ − mean $(P_{3/4})$) of [deoxy-Hb] was 0.051±0.02 a.u. The presence of this post-stimulus overshoot was shown to be statistically significant ($p < 0.05$, one- sample *t*-test, 9 d.f., see table 3), too.

Figure 4 compares $A_{2\text{-}10} - A_1$ (initial undershoot) and $P_{1/2} - P_{3/4}$ (post-stimulus overshoot). The numbers refer to tables 1 and 3 and to the examples given in figure 3. Bivariate correlation analysis showed a significant correlation ($r = 0.799$, $p < 0.01$, Pearson's product moment). The regression line is described by the term $y = 0.92x - 0.025$.

Table 3. *Mean [deoxy-Hb] of ($A_{2-10}-A_1$)—initial undershoot—and mean [deoxy-Hb] of ($P_{1/2}-P_{3/4}$)—post-stimulus overshoot—for the ten included subjects, numbers correspond to table 1 and figure 3*

(Mean [deoxy-Hb] (±s.e.m., a.u.) and significance are given (one-sample *t*-test, two-tailed, compared to 0). Column 3 displays the ratio of initial undershoot and post-stimulus overshoot.)

subject	mean (A_{2-10}) − mean (A_1)/a.u. initial undershoot	mean (P_{1-2}) − mean (P_{3-4})/a.u. post-stimulus overshoot	ratio initial undershoot to post-stimulus overshoot
#1	0.159	0.064	2.48
#2	0.090	0.072	1.25
#3	0.027	−0.018	−1.54
#4	0.128	0.047	2.72
#5	0.157	0.182	0.86
#6	0.112	0.099	1.12
#7	0.068	0.082	0.84
#8	0.005	−0.010	−0.52
#9	0.032	−0.015	−2.07
#10	0.055	0.011	4.98
mean	0.083	0.051	1.01
s.e.m.	0.016	0.019	0.62
sig. (one-sample *t*-test)	$p = 0.001$	$p = 0.050$	

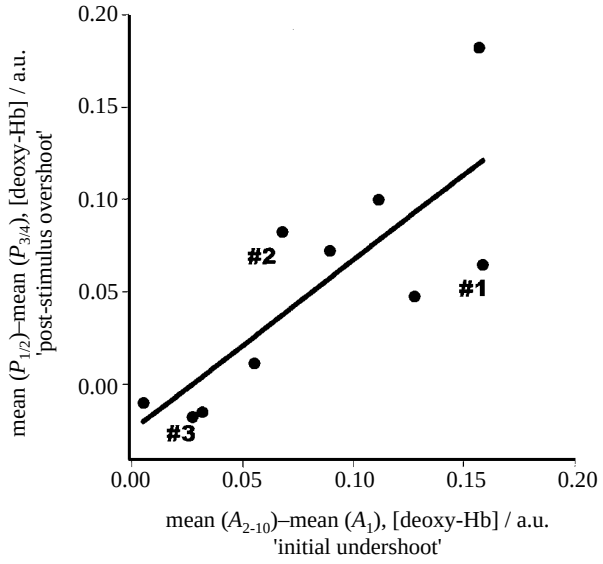

Figure 4. Correlation of initial undershoot (*x*-axis), of [deoxy-Hb]($A_{2-10}-A_1$) and post-stimulus overshoot (*y*-axis), of [deoxy-Hb] ($P_{1/2}-P_{3/4}$), $r = 0.799$ (Pearson's product moment), $p < 0.01$; the regression line is described by the term $y = 0.920 \times -0.025$. Numbers refer to tables 1 and 3, and to the traces in figure 3.

4. DISCUSSION

In agreement with fMRI and PET findings (Fox *et al*. 1986; Frahm *et al*. 1992; Kwong *et al*. 1992; Ogawa *et al*. 1992) in previous NIRS studies (for a review see Obrig & Villringer (1997)), we have demonstrated a typical response pattern to functional brain activation that consists of a decrease in [deoxy-Hb] and an increase in [oxy-Hb] (Villringer *et al*. 1993; Obrig *et al*. 1996; Wenzel *et al*. 1996). The amount of change reported here is in line with previous findings by our and other groups (Meek *et al*. 1995; for a review see Obrig & Villringer (1997)), and are much smaller than the probably maximal changes occurring during occlusion of the internal carotid artery in piglets (Matcher *et al*. 1995). In the present study, we have shown that, (i) during prolonged visual stimulation with a stationary dodecahedron in most subjects the decrease in [deoxy-Hb] gradually returns to a value closer to the baseline: the initial pronounced decrease in [deoxy-Hb] may be regarded as an initial undershoot, (ii) after cessation of stimulation there is a post-stimulus overshoot of [deoxy-Hb], (iii) the size of the initial undershoot and the post-stimulus overshoot are positively correlated, and (iv) [oxy-Hb] increases during functional activation and remains constantly elevated throughout the stimulation period with no initial overshoot. After cessation of the stimulation there is a post-stimulus undershoot.

Subsequently, we discuss these findings in the light of recent fMRI studies employing the BOLD-contrast. It is generally assumed that the BOLD signal is inversely related to [deoxy-Hb]. We are aware of certain shortcomings of NIRS in its present implementations as compared to fMRI. These include its poorer spatial resolution, the potential for extracerebral signal contamination, and the inability to differentiate between the contribution of small or large vessels (Obrig & Villringer 1997). However, the main advantage of NIRS is its biochemical specificity, which gives a much more direct measurement of [deoxy-Hb] than the indirect BOLD-contrast.

When comparing the behaviour of [deoxy-Hb] in the present study to the time-course of the BOLD signal as reported in fMRI studies there is general agreement that within the first 20 s of localized brain activation a drop in [deoxy-Hb] occurs, associated with an increase in the BOLD signal. Within this temporal window, indeed in a previous simultaneous fMRI–NIRS study, we have shown a spatial congruence of the BOLD signal increase and the [deoxy-Hb] drop (Kleinschmidt *et al*. 1996).

Another point of agreement concerns the post-stimulus overshoot for [deoxy-Hb] as seen by NIRS, which is seen in the BOLD signal as a post-stimulus undershoot.

Controversy exists in the fMRI literature, however, concerning the presence of an initial BOLD overshoot and a subsequent return of the BOLD signal to values much closer to the baseline. The time-course of [deoxy-Hb] in this study does show an initial undershoot in the grand average and in seven out of ten individual subjects. This is in line with data by Frahm *et al.* (1996), Krüger *et al.* (1996), and Hathout *et al.* (1994), who showed a similar (reverse) time-course of the BOLD signal in fMRI. However, other groups have shown a constantly elevated BOLD signal throughout the activation period. Although our data clearly indicate that an initial undershoot does occur at least in certain conditions, presently, we have no final explanation for the discrepancy between the fMRI studies. It has been proposed that the difference between the various fMRI studies may be due to different technology, such as using echoplanar versus FLASH-type pulse sequences. However, in a recent study, Howseman *et al.* (1996) showed no difference in the time-course of fMRI signal intensity with both types of MR-pulse sequences. Therefore, the most likely explanation seems to be in the different stimuli employed by the different groups. Frahm *et al.* (1996) used a flickering pattern, Krüger *et al.* (1996) employed presentation of a video, Bandettini *et al.* (1995) used checkerboard stimulation, and Howseman (1996) also used a checkerboard stimulation protocol. An influence of stimulus modality is also indicated by the fact that during motor activation there is no oxygenation overshoot as all authors agree. In order to clarify the influence of the stimulation protocol further we are currently performing experiments employing different activation paradigms.

To clarify the time-course of haemoglobin oxygenation changes during prolonged stimulation is important with respect to both the interpretation of fMRI studies and the underlying physiology. One interesting interpretation of the initial oxygenation overshoot may be a transient pronounced decoupling of CBF and oxidative metabolism (Frahm *et al.* 1996). The subsequent plateau of [deoxy-Hb] may represent a new equilibrium between the two, caused by a delayed increase in oxygen consumption. In this theoretical model the post-stimulus [deoxy-Hb] overshoot would reflect the reverse of the initial [deoxy-Hb] undershoot. The statistically significant positive correlation between these two events which we observed in this study would fit well with this model.

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