laughter. During the post war years, he was joined by the artist Raymonde Parsons and one cannot dissociate the two. She created a background of support and comfort for J.Z. Young and his associates which was an integral part of this period of great scientific productivity.

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TECHNIQUES

Non-invasive optical spectroscopy and imaging of human brain function

Arno Villringer and Britton Chance

Brain activity is associated with changes in optical properties of brain tissue. Optical measurements during brain activation can assess haemoglobin oxygenation, cytochrome-c-oxidase redox state, and two types of changes in light scattering reflecting either membrane potential (fast signal) or cell swelling (slow signal), respectively. In previous studies of exposed brain tissue, optical imaging of brain activity has been achieved at high temporal and microscopical spatial resolution. Now, using near-infrared light that can penetrate biological tissue reasonably well, it has become possible to assess brain activity in human subjects through the intact skull non-invasively. After early studies employing single-site near-infrared spectroscopy, first near-infrared imaging devices are being applied successfully for low-resolution functional brain imaging. Advantages of the optical methods include biochemical specificity, a temporal resolution in the millisecond range, the potential of measuring intracellular and intravascular events simultaneously and the portability of the devices enabling bedside examinations.

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IT IS WELL KNOWN and widely used in medicine that the functional state of tissue influences its optical properties. Cyanosis indicates poor tissue oxgenation, paleness may be related to anaemia and yellow colouring may be due to increased bilirubin concentration indicating liver failure. For the nervous system, it was reported as early as 1949 that the activity of nerve cells was associated with changes in their optical properties[1]. Since then, changes in optical properties of brain cells have been reported in cell cultures[2], bloodless brain slices[3,4], as well as in intact cortical tissue[5–7]. Studies have been performed with and without contrast agents (extrinsic versus intrinsic optical signals). Optical signals have been used to map brain function after surgical exposure of cortical tissue in animals[5] and human subjects[8]. Recently, it has been shown that it is possible to assess brain activity through the intact skull in adult human subjects[9–13] and even non-invasive functional brain mapping[14–17] has become possible.

Functional optical imaging is the assessment of physiological changes associated with brain activity by optical methods. In order to understand how functional optical maps of brain activity are generated, we subsequently describe: (1) the interactions of photons with brain tissue which may be measured by optical methods (optical parameters); (2) the physiological events which are associated with brain activity (physiological parameters); and finally (3) the relationship between optical and physiological parameters.

Optical parameters

Photons that enter tissue may undergo, in principle, the following types of interaction with tissue (please note that there are other interactions, for example, Raman effects which are not covered here): (1) **absorption** which may lead to radiationless loss of energy to the medium, or induce either **fluorescence** (or delayed fluorescence) or **phosphorescence**; and (2) **scattering** at unchanged frequency when occurring in stationary tissue or accompanied by a **Doppler shift** due to scattering by moving particles in the tissue (for example, blood cells).

Typically, an optical apparatus consists of a light source by which the tissue is irradiated, and a light detector that receives light after it has been reflected from or transmitted through the tissue. Many sender–receiver pairs are used in imaging devices[16,18,19]. Light that has travelled through tissue is attenuated mainly due to absorption and scattering. By analogy

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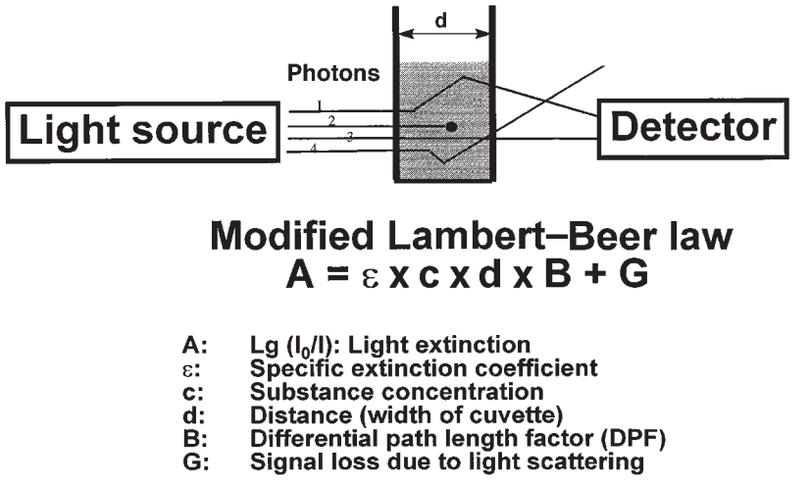

Fig. 1. ***Influence of light absorption and scattering on optical measurement: modified Lambert–Beer law.*** *The concentration of a light absorbing molecule in tissues is determined similarly to the determination of a substance concentration in a photometer. Assuming infinitesimal substance concentrations, and therefore neglible light scattering, a concentration can be determined according to the original Lambert–Beer law, $A = \epsilon \times c \times d$, in which the extinction of light [the logarithm of the ratio of incident versus measured light, $lg(I_0/I)$] is proportional to the concentration (c) of the absorber multiplied by the constant extinction coefficient (ϵ) for the particular absorber and the distance (d) corresponding to the width of the cuvette. This law holds as long as photons are either absorbed (photon 2) or transmitted in a straight line directly to the detector (photon 3). With higher substance concentrations and significant light scattering, the formula must be modified to take into account the longer pathlength of light (see photon 1) and the loss of light (photon 4) due to light scattering. In the modified Lambert–Beer law as given in the Figure, therefore a term B which accounts for the longer pathlength and a term G, which is a measure of the signal loss due to light scattering and which depends mainly on geometrical factors are introduced. In certain situations only the difference (Δc) between two situations is of interest and under the assumption of a constant light scattering loss the term G cancels out in the subtraction. $\Delta A = \varepsilon \times \Delta c \times d \times B$. If the pathlength $d \times B$ can be determined, absolute changes in concentration can be calculated. For absolute measurements of the pathlength there are several types of optical approaches. One uses the measurement of the direct time of flight of a short (ps) light pulse travelling through tissue[20]. In another approach the phase shift of a light source which is intensity-modulated at a certain frequency is measured[21]. A third approach measures water absorption which, assuming a constant water concentration in tissue, should change with the pathlength of light[22]. The above mentioned approaches for the determination of pathlength may not only serve for a more accurate determination of a substance concentration, but they may also serve to measure light scattering (or changes in light scattering) as another optical parameter.*

with a photometer, this can then be expressed mathematically in a modified Lambert–Beer law (Fig. 1).

Separating light of different wavelengths using certain filter designs is the basis for the detection of fluorescent or phosphorescent light which has a longer wavelength than the irradiated (and reflected) light. The detection of the Doppler frequency shift of scattered light is the basis of Laser Doppler Flowmetry (LDF) devices[23].

Physiological processes associated with brain activity

Physiological events associated with brain activity can be subdivided into those that occur intracellularly or at cell membranes and those that are mediated by neurovascular coupling and occur within the vascular space.

Cellular physiological events

The activity of neurones is characterized by ion and water fluxes across the neurone's membrane inducing a change in membrane potential, as well as electrical and magnetic field changes. The main ions involved are Na^+, K^+, Cl^- and Ca^{2+} with the ion shifts inducing changes in their intracellular and extracellular concentrations. Furthermore, second messenger systems, such as cAMP (Ca^{2+} itself also serves as a second messenger) are activated.

Increased activity of brain cells is associated with an increase in glucose consumption and oxygen consumption (but see Ref. 24). Intracellular glucose concentration drops at least transiently[25,26] with a transient rise in local lactate concentration[27,28]. It is possible, that these events also affect the redox state of intracellular NADH, flavoproteins and cytochrome-*c*-oxidase (CO); early data by Chance and Williams have indicated that during increased activity these are generally oxidized[29]. Subsequent work in intact animals has indicated increased oxidation of NADH[30] and CO (Refs 31,32) during brain activation, and recent data in human subjects also indicate increased CO oxidation[33]. However, due to the potential interference of the oxy-Hb signal and the lack of a gold standard for CO measurements this is still a controversial issue[34].

Intravascular events

In addition to those events taking place intracellularly, local brain activity induces a local arteriolar vasodilation and consequently an increase in local cerebral blood volume (CBV) and blood flow (CBF), termed neurovascular coupling[35] (for a recent review see Ref. 36). At the capillary level, the increase in CBF is achieved mainly by higher blood flow per capillary, associated with higher blood flow velocity rather than with opening and closing of previously unperfused capillaries. The increase in CBF and oxygen delivery exceeds the increase in local oxygen consumption[24]. Therefore, cerebral blood oxygenation rises locally[36].

Relationship between brain activity and optical parameters: intrinsic optical signals

Optical measurements are classified as either extrinsic (using exogenous contrast agents) or intrinsic (without exogenous contrast agents). The relationship that each has with brain activity is illustrated in Fig. 2.

Light scattering and cell activity

Light scattering occurs at borders of media with different refractive indices. Therefore, it seems plausible that events occurring in neuronal membranes and volume changes of cellular compartments or of organelles associated with brain activity can influence light scattering. Activity-related light scattering changes have been measured in isolated axons[1], neuronal cell cultures[2], brain slices[3,4], the neurohypophysis *in vitro*[37], as well as in intact animals[38,39]; recently, even non-invasive measurements in human adults have been reported[13].

Two types of light scattering signals have been reported associated with brain cell activity:

(1) A fast signal[2,37] which has response times on the order of probably less than a millisecond and which may be related to changes in the index of refraction at neuronal membranes. In cultured neurones this light scattering signal correlates linearly to the change in membrane potential, and axon potentials can be observed[2] (see Fig. 3A). This fast signal may be the physiological explanation for the non-invasively measured fast optical signals in human subjects[13].
(2) A slower scattering signal with response times in the order of a few seconds has been described in hippocampal brain slices as well as in intact animals[4,38]. MacVicar and Hochman have given evidence that these signals might be related to high $[K^+]$-associated glial swelling[4].

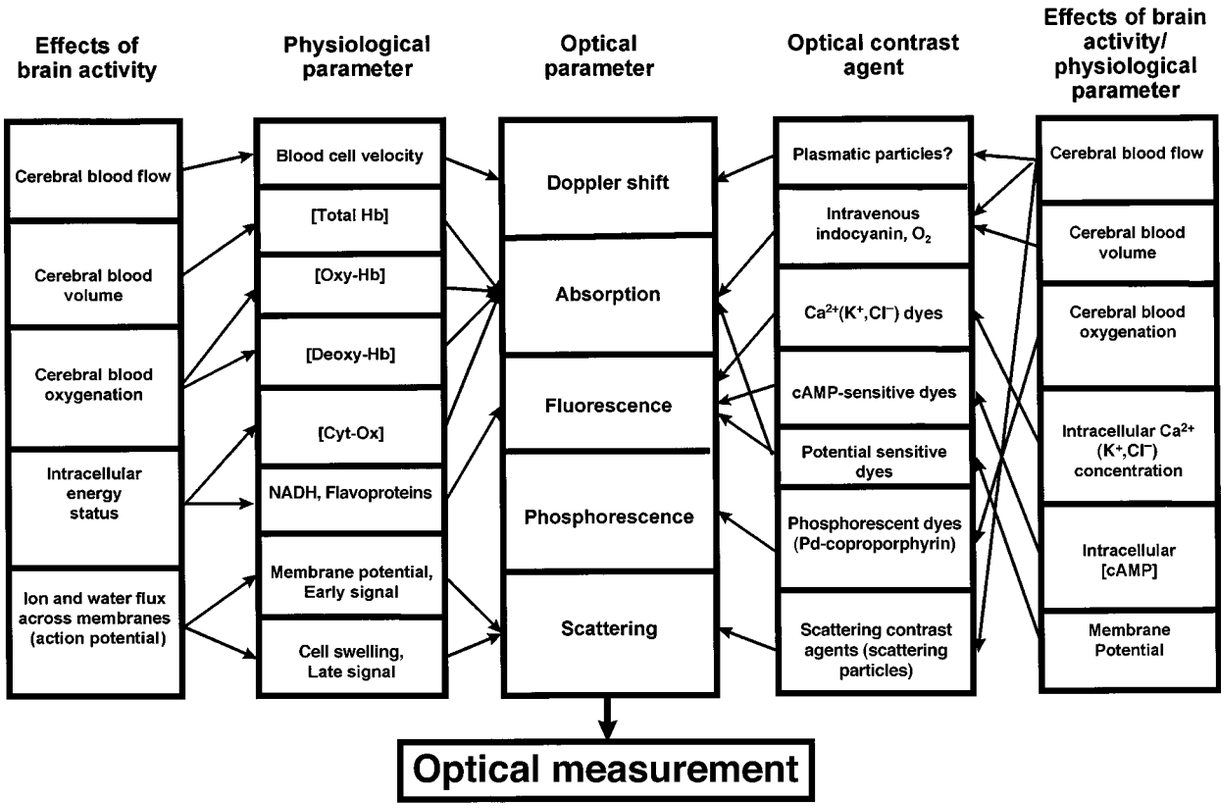

Fig. 2. ***Assessment of brain activity by measuring optical parameters.*** *The left part of the figure illustrates the relationship between brain activity and optical measurements based on intrinsic optical contrast. Brain activity is accompanied by certain physiological events, for example, an increase in cerebral blood oxygenation. These events influence the value of certain intrinsic physiological parameters, for example, the concentration of oxy-Hb, that, in turn, can be measured through their influence on optical parameters, for example, light absorption. The right part of the figure illustrates the relationship between brain activity and optical measurements employing exogenous contrast agents (extrinsic optical signals). Brain activity is accompanied by certain physiological events, for example, an increase in* $[Ca^{2+}]_i$ *(physiological parameter). Certain contrast agents, for example calcium-sensitive fluorescent dyes, change their optical properties (fluorescence) according to this physiological parameter and the optical parameter (in this case fluorescence) is then assessed with the optical apparatus. The term physiological parameter is used to indicate a physiological quantity which is measured by its correspondence to an optical parameter.*

Intracellular energy status and brain activity

NADH oxidation as well as flavoprotein oxidation which can be measured with fluorescence techniques[41] seem to change towards more oxidation during brain activation and spreading depression[29,30]. CO has a characteristic absorption pattern that is influenced by its redox state and therefore, the redox state of CO can be measured using visible light and near-infrared light[42]. Studies on the exposed brain of rats during local electrical stimulation indicated a transient increase in CO oxidation[31,32] hence, CO redox state may serve as an intracellular marker of brain activity.

Cerebral blood flow, oxygenation and brain activity

Cerebral blood flow changes associated with brain activity have been measured with several optical techniques. In measurements of the surface of brain tissue[43], LDF takes advantage of the Doppler shift of photons meeting moving blood cells and it has been shown that the relative LDF measurements correlate with other CBF measurements[44–46]. During increased brain activity, within several seconds, there is an increase in local cerebral blood flow[47]. The combination of scanning devices and Laser Doppler technology allows the surface imaging of changes in brain activity[48]. The increase in CBF exceeding the increase in oxygen consumption leads to an increase in intravascular haemoglobin oxygenation during brain activity. Oxygenated and deoxygenated haemoglobin (oxy-Hb, deoxy-Hb) have characteristic absorption patterns in the visible and near-infrared light range. Therefore based on light absorption measurements, concentration changes of these molecules can be measured during functional brain activation[9–12,38].

Extrinsic optical signals and brain activity

This review focuses on non-invasive approaches without contrast agents. It should be mentioned, however, that in many circumstances, using contrast agents means the amount of physiological information to be obtained with optical methods can be increased significantly (see right side of Fig. 2). Dyes have been developed that can be loaded intracellularly and change their fluorescent or absorption behaviour depending on membrane potential[49] or the concentration of a variety of different ions such as Ca^{2+}, Mg^{2+}, K^+, Cl^- and even second messengers such as cAMP (Refs 50,51). On the intravascular side, similar to positron emission tomography (PET) or functional magnetic resonance imaging (fMRI), indicator dilution approaches can be used to measure cerebral transit time and cerebral blood flow[52–55] and such CBF measurements can then be used to indicate changes in brain activity. Similarly, cerebral blood oxygenation changes can be assessed by measuring phosphorescence of intravascularly placed dyes[56,57] and theoretically this method could also be used for monitoring of brain activity.

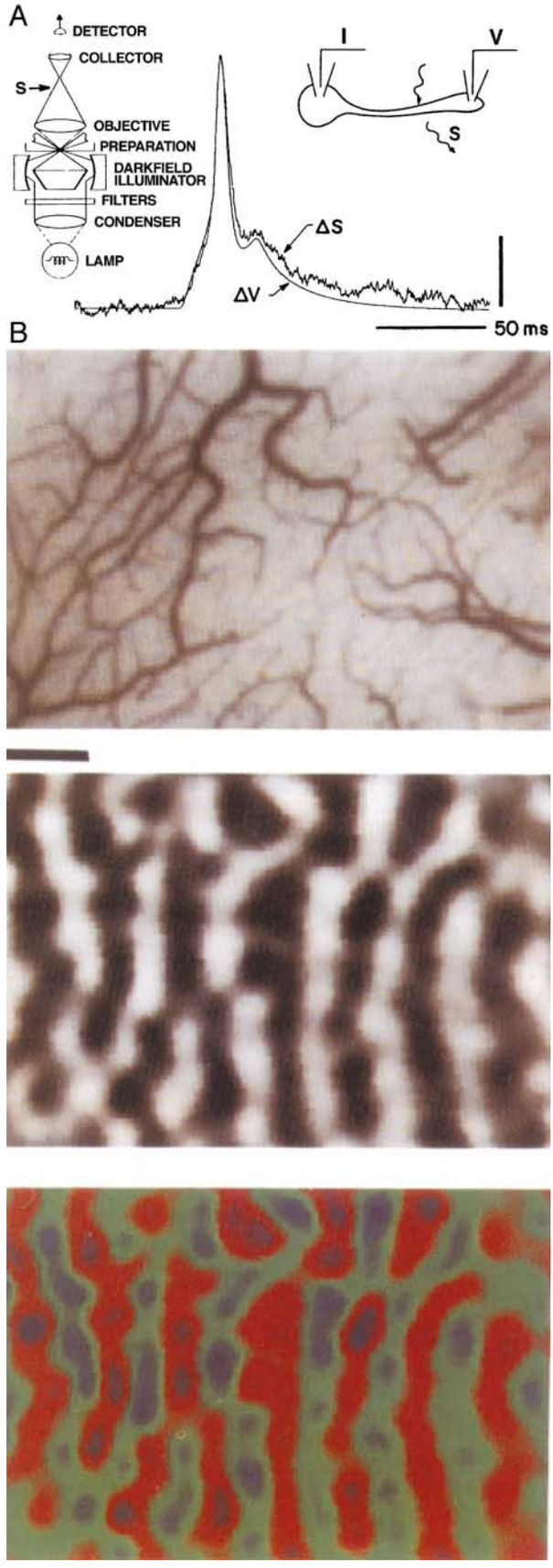

Fig. 3. ***Intrinsic optical signals and their measurements.*** **(A)** *Measurement of electrical activity by the detection of scattered light in a microscope equipped for dark-field illumination. In the axonal stump (inset upper right) of a neurone in a neuronal culture, light scattering (ΔS) was measured simultaneously with electrical intracellular recording (ΔV). Data are the average of 500 traces. The inset on the left is a schematic diagram of the optical apparatus.* **(B)** *Imaging of ocular dominance columns using intrinsic optical signals. Intrinsic optical signals measured in the occipital cortex of an awake monkey. The top image gives a picture of the imaged area. The middle image gives the ocular dominance map. This map was obtained by dividing 48 cortical images taken when the right eye was viewing the video movie 'Winnie the Pooh' by 48 cortical images taken when the left eye viewed the movie. The image on the bottom is the same as the middle one, except that a pseudocolour map was used. The intensity of the red denotes the dominance by the left eye and intensity of the green denotes the dominance by the right eye. The blue regions correspond to the centre of the highest monocular activity and lie in the centre of each ocular dominance column. Scale bar = 1 mm.* Reproduced from Refs 2,40.

Functional brain mapping: results of invasive optical measurements

The type and quality of information that optical methods can supply is shown very impressively by methods relying on the direct exposure of brain tissue to the optical apparatus. Using membrane potential-sensitive dyes loaded into brain cells, activity of individual cells can be monitored at a temporal resolution in the order of milliseconds and at microscopic spatial resolution[49]. Even without the application of dyes, highly interesting functional information can be obtained by measuring the light reflected from brain tissue. Without further separation of the signal into its physical or physiological components (absorption, scattering, [deoxy-Hb], [oxy-Hb]), mapping of activated brain areas in the visual cortex is possible (intrinsic optical signals) at high temporal and spatial resolution[5,40] (see Fig. 3B). Such signals can also be obtained in human subjects during brain surgery[8]. The method of optical coherence tomography (OCT) may provide additional spatial resolution to this surface approach which is probably limited to the outer millimeter of the brain[58].

In non-invasive measurements, these optical signals are distorted by the multiple scattering that occurs when the light travels twice across extracerebral tissue. It is therefore the goal of non-invasive optical approaches to extract this information as well as possible.

Non-invasive optical approach of near-infrared spectroscopy (NIRS)

Biological tissue is relatively transparent to light in the near-infrared range between 700–1000 nm. This is due to the fact that water absorption and hemoglobin absorption are relatively small within this wavelength region. Therefore this wavelength range represents an 'optical window' for the non-invasive assessment of brain tissue[59]. The light source is coupled to the subject's head via fibre-optical bundles (optode). Since light is highly scattered after entering tissue a second optode, placed 2–7 cm away from the first can collect light after it has passed through the tissue beneath the optodes. The light-receiving optode is connected to a light detecting system such as a photomultiplier or a CCD camera.

Several models of the sample volume have been proposed based on different assumptions and modelling algorithms. In early attempts, a homogeneous tissue beneath the optodes was assumed and according to these model calculations the sample volume corresponded to a banana-shaped volume[60] beneath the optodes and the depth of penetration seemed mainly to be a function of the interoptode distance. In recent more sophisticated approaches, a multi-layer model imitating skin, skull, CSF and brain tissue has been

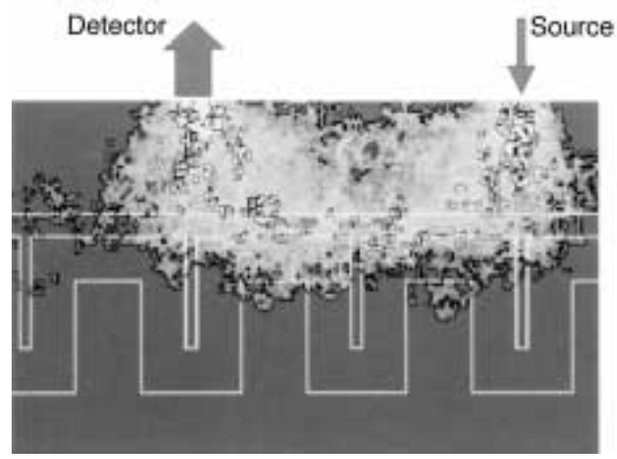

Fig. 4. ***Near-infrared spectroscopy and imaging.*** *The spatial sensitivity profile of photons travelling in a 'sophisticated' (including sulci) four-layer model of the adult human head. The layers are: (1) surface (scalp and skull); (2) cerebrospinal fluid; (3) grey matter and (4) white matter The distance between the light source and the detection fibre is 30 mm.* Reproduced from Ref. 61; see also Ref. 62 for data on the neonatal head.

assumed[61]. In such models the important role of the CSF layer with its potential 'light tunnelling' properties has been emphasized by the Monte-Carlo Method employed. Based on such models the shape of the sample volume has to be modified (see Fig. 4). Interestingly, however, hematomas located 4 cm beneath the surface as shown by a CT-scan of a patient, have been detected with NIRS indicating the potential for even deeper penetration of near-infrared light[65].

It seems that photons spend a considerable amount of time in non-cerebral tissue such as skin, skull and CSF (these tissues, however have a smaller blood and cytochrome content than brain tissue). An interoptode distance that was too small did result in unacceptably high extracerebral contamination in some studies[66]. Studies by Kirkpatrick's group on the other hand have shown that extracerebral contamination can be minimized with careful setup of the measurement[67]. However, contribution of extracerebral tissue to the signal remains a major issue to be addressed. Ideally, optical systems should try to differentiate between the contributions from cerebral and extracerebral tissue, for example, by measuring distributions of photon pathlengths. If, however, depth resolution of the signal is not possible, as in most current commercially available NIR systems, only measurements during which it can be reasonably assumed (or measured) that there is no pronounced change in extracerebral blood flow or oxygenation, or both, can be attributed to brain tissue.

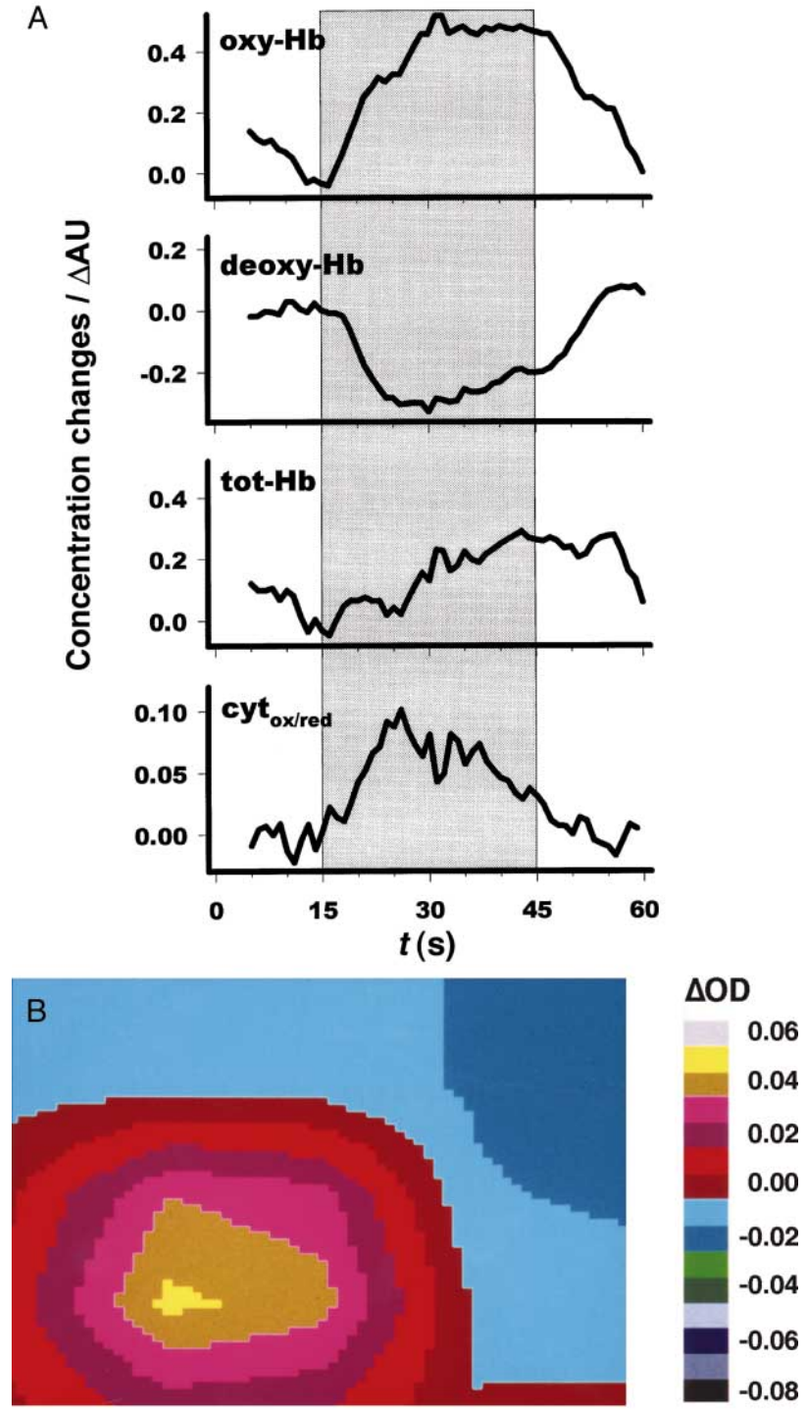

Fig. 5. ***Near-infrared spectroscopy and functional imaging during visual stimulation.*** **(A)** *Typical changes in hemoglobin oxygenation and CO oxidation in the occipital cortex during visual stimulation as measured with a four-wavelength near-infrared spectroscopy system (NIRO 500 system[63]) in a healthy human volunteer. According to an anatomical MRI, the optodes were located over the occipital cortex[64]. The interoptode distance was 3.5 cm. The grey-shaded area depicts the 30 s of visual stimulation that was interleaved with a rest period of equal length. Changes in [oxy-Hb], [deoxy-Hb], [tot-Hb] and [$CO_{ox/red}$] are given in arbitrary units (AU), which correspond to μM concentration changes when assuming a DPF (differential path length factor) of 6.26 (Ref. 21). Note that the range of the Y-axis for [oxy-Hb], [deoxy-Hb] and [tot-Hb] is 0.6 AU, whereas for [$CO_{ox/red}$] it is 0.15 AU.* **(B)** *Functional near-infrared imaging during visual stimulation. Focal increase in blood volume (corresponding to [tot-Hb]) in response to visual stimulation as measured over the right occipital cortex with a functional NIRS imaging device[16,19]. The imaging device consists of an array of nine light sources and four detectors resulting in 16 sender-receiver pairs covering an area of 9 × 4 cm. The system uses white light sources and filters are placed in front of the PMT to let wavelengths 760 nm and 850 nm pass. For functional imaging, the imaging array was placed over the right occipital lobe of a healthy human volunteer. Visual stimulation consisted of 40 s of observation of a stationary dodecahedron interleaved with a 40 s period of rest. The area of the peak response is clearly focussed, about 0.5 × 0.5 cm of size, the average amount of change is 0.2 OD. The point of maximum signal increase during the visual stimulation projects to a point situated about 2.5 cm laterally of the midline and about 1.5 cm above the inion (for more detail see Ref. 17).*

The major intracerebral contribution probably comes from the grey matter[61]. This has been confirmed in two studies performing PET and NIRS simultaneously that have shown the best correlation between NIRS and PET parameters in the outer 1 cm of the brain tissue[68,69]. Interestingly, it seems that even at inter-optode distances as short as 2–2.5 cm grey matter is part of the sample volume[61]. This is consistent with work assessing changes in local brain activity successfully with inter-optode distances of 2.5 cm (Ref. 11). Other authors have reported measurements at even smaller inter-optode distances[10].

Near-infrared spectroscopy and brain activity

Several NIRS studies in recent years have demonstrated that changes in brain activity can be assessed non-invasively in adult human subjects[9–17]. Several types of brain activity have been assessed, including motor activity[14,15,70], visual activation[11–13,64,71], auditory stimulation[10] and performance of cognitive tasks[9,10,12]. NIRS-parameters (oxy-Hb, deoxy-Hb, CO) exhibit typical responses to functional brain activation using a four-wavelength system (Fig. 5A). Most studies, until recently, were performed with single-site NIRS systems, but recently several studies have shown that multisite mapping of brain activity is also possible[10,14,15]. Multi-optode arrays[18] are now being developed that have been shown to permit functional neuroimaging[16,17] (see Fig. 5B).

In principle, all optical parameters (given in Fig. 2) can be measured. However, without the use of exogenous contrast agents no significant fluorescent or phosphorescent signal can be detected transcranially. Whereas in surface measurements LDF is a very reliable technique for the measurement of CBF (Ref. 45), no transcranial analogue has yet been developed.

Based on assessment of absorption and scattering, three types of activity-related signals have been recorded non-invasively: (1) changes in haemoglobin oxygenation; (2) changes in CO oxidation; and (3) fast optical signals presumably related to changes in light scattering.

Haemoglobin oxygenation

Studies on exposed brain tissue[38] suggest that increased brain activity is associated with an early decrease in haemoglobin oxygenation followed by a subsequent longlasting increase in haemoglobin oxygenation. Near-infrared studies have focused so far on the latter slower response and several studies have measured non-invasively the increase in haemoglobin oxygenation that occurs within several seconds after the onset of increased brain activity[10–12]. Similar to the blood oxygenation level dependent (BOLD) contrast in functional magnetic resonance imaging (fMRI)[72–74] these signals reflect the fact that the blood flow response to functional activation is larger than the increase in oxygen consumption[24]. Therefore, when the NIRS measuring site is located over an area in which cerebral blood flow increases during brain activity, for example, the occipital cortex during visual stimulation, a localized increase in [oxy-Hb] and a decrease in [deoxy-Hb] is seen[64] (Fig. 5A). Consistent with this notion, in simultaneous PET–NIRS studies, during stimulation tasks positive correlations between Δ[oxy-Hb] and ΔCBF, and between Δ[total-Hb] and ΔCBF along with a negative correlation between Δ[deoxy-Hb] and ΔCBF were observed[68,69] (an earlier PET study on two subjects had also shown agreement between the direction of changes in CBF and total haemoglobin[75]). Furthermore, in a study employing simultaneous NIRS and BOLD–fMRI (a signal inversely related to [deoxy-Hb]), a good spatial agreement between the BOLD measurement and the drop in [deoxy-Hb] by NIRS was noted[76]. Based on the measurement of hemoglobin oxygenation changes, employing recently developed multi-optode arrays[16,19], it has become possible to create non-invasive functional optical images of human brain activity (see Fig. 5B). Recently, comparisons of the functional images acquired with such near-infrared imaging devices and BOLD–fMRI have resulted in good spatial congruence of the two modalities[16].

Whereas the response of haemoglobin oxygenation to increased brain activity has been studied extensively, little is known about what happens when a certain brain area becomes deactivated. A pronounced deactivation (that is, decrease in CBF) of the occipital cortex during vestibular stimulation has been reported by Wenzel *et al.*[77], and recently, it has been shown that, during performance of accoustically cued saccades in darkness, a similar deactivation occurs in the occipital pole. Under these conditions, NIRS shows a rise in [deoxy-Hb] and a drop in [oxy-Hb], hence the reverse behaviour as seen in cerebral activation. Deactivation phenomena in the frontal cortex during performance of mental tasks have also been described in a simultaneous PET–NIRS study[75].

CO redox state

CO redox state would be a very interesting marker of intracellular energy metabolism; however, near-infrared CO measurements are the most difficult to interpret (for a recent review see Ref. 42). Measurements on exposed brain tissue using visible light have indicated a transient oxidation of CO during electrical stimulation[31,32]. Using non-invasive continuous wave NIRS systems similar findings of transient oxidation are obtained reproducibly (see Fig. 5A). However, operating at only four wavelengths it seems difficult to exclude crosstalk of the much stronger oxy-Hb-signals as a potential source of error in these CO measurements. Improved spectral resolution using a NIRS approach employing a CCD camera[22] may make CO oxidation measurements more reliable in the future. Our results using this improved method still indicate increased oxidation of the (presumably already highly oxidized) CO with increasing brain activity in human subjects[33]. Further optimization of algorithms and validation experiments are needed to solve the still ongoing controversy regarding these CO measurements[34].

Light scattering

Using a phase-modulated near-infrared system, Gratton *et al.* have reported a transient increase in pathlength of light in the occipital cortex with a maximum at 100 ms after the onset of visual stimulation[13]. It seems very unlikely that the concentration of an absorbing molecule has changed so shortly after onset of brain activity to such an extent that this induces such pronounced pathlength changes, especially since these early pathlength changes are larger than those induced by the subsequent changes in haemoglobin concentration and oxygenation. Therefore, it seems reasonable to assume that such a signal might correspond to a transient change in light scattering similar

to the signals obtained by Stepnoski *et al.* on single neurones[2]. These findings[13], however, still await confirmation by other groups. At later time points after the onset of functional activation, the separation of scattering and absorption contributions to the optical signal is difficult, especially in a non-invasive study. As yet (except for the invasive study by Malonek and Grinvald[38]), no convincing measurement of such changes in light scattering has been reported.

Pathological response to brain activation and response to pathological brain activation

So far we have reviewed the typical changes in optical parameters during brain activity under normal physiological conditions in young and healthy human subjects. The amplitude of the vascular response seems to decrease in an age-dependent way as suggested by two NIRS studies[78,79]. It is not clear yet whether this decrease is concomitant with subclinical vascular disease or is indeed just age-dependent. The finding seems to be consistent with PET studies indicating that resting $CMRO_2$, CBF and CBV decrease with age[80].

A number of experimental data in animals[81] as well as preliminary clinical data[82] indicate that cerebrovascular disease can significantly influence the blood flow and oxygenation response to functional activation and this abnormal response should reflect in NIRS studies. Preliminary data have shown alterations in NIRS responses and in PET measurements taken simultaneously in subjects with Alzheimer disease[68].

In addition to physiological brain activity induced by visual, somatosensory or other types of brain activation there are also pathological types of altered brain activity. Most notably, pathological brain activity is present during epileptic seizures. Optical studies in rats have indicated increased haemoglobin oxygenation during seizures elicited by bicucculine[83] and pentylentetrazol[84]. Our NIRS measurements obtained during complex-partial seizures and taken as close as possible to the presumed focus in the frontal cortex of human adults also indicate an increase in [oxy-Hb] during seizure. In contrast, when performing NIRS measurements in the prefrontal cortex during absence seizures, there was a consistent drop in the concentration of [oxy-Hb] and an increase in the concentration of [deoxy-Hb] (Ref. 85).

Another type of pathological brain activity that is known experimentally as spreading depression (SD) might be a correlate of the aura in migraine. SD is associated with an increase in [oxy-Hb] (Refs 86, 87), a decrease in [deoxy-Hb] (Ref. 87) and large changes in light scattering[39]. A variant of SD occurs in the penumbra of experimentally-induced infarctions, peri-infarct depolarizations (PID) and is probably an important mechanism for irreversible brain damage occurring to the penumbra[88]. During PID, NIRS measurements have shown a drop in [oxy-Hb] and a rise in [deoxy-Hb] consistent with transient deoxygenation[89]; it is very likely that there are also large changes in light scattering. The response of NIRS parameters to these different types of pathological brain activity can be inferred from animal studies (Table 1). These properties currently serve as 'footprints' for the non-invasive detection of similar events in human subjects (especially in patients with migraine, stroke and head trauma).

Human applications of NIR-spectroscopy and imaging

The method of NIR-spectroscopy and imaging is still in the early phase of applications in the neurosciences. Compared to other functional neuroimaging methods such as PET and fMRI it lacks, in particular, spatial resolution and depth penetration, limiting most current studies to the cortical grey matter. Furthermore contributions from extracerebral tissue may contaminate the signal in non-imaging NIR devices. Absolute determination of concentrations is difficult without implementation of more sophisticated approaches, such as time-resolved NIRS. Therefore, most current studies can report only on concentration changes. On the other hand, the NIR methods do have some unique properties that may offer advantages over other methods. In contrast to other techniques, in particular fMRI, NIRS offers biochemical specificity by measuring concentrations of biochemically well defined substances such as oxy-Hb, deoxy-Hb and CO redox state. Whereas other functional neuroimaging methods such as fMRI, PET, EEG (electroencephalography) or MEG (magnetoencephalography), measure either correlates of neuronal activity (EEG, MEG) or the vascular response to it, optical methods, in principle, can assess both, not only aspects of the vascular response such as oxy-Hb and deoxy-Hb but also intracellular events (CO redox state, light scattering). Optical methods have intrinsically good temporal resolution limited just by the signal to noise. Even in transcranial examinations in human subjects a temporal resolution in the millisecond range seems feasible. Since optical systems are usually flexible and portable, bedside examinations are easily feasible, enabling patients to be examined repetitively or monitored continuously for extensive time periods. Finally, the cost of even highly sophisticated time-resolved NIR imaging systems of the future will be far less than those of other functional neuroimaging devices.

Based on the current status of the developments in optical imaging and considering relative advantages and disadvantages as compared to other functional neuroimaging methods, a number of applications are envisioned for optical methods. In basic neuroscience, optical methods are a unique approach to study neurovascular coupling, since both ends of this process (the neuronal and the vascular) can be measured simultaneously. In cognitive neuroscience optical methods may be useful in localizing brain activity, particularly in situations in which other methods are not applicable. For example, optical measurements could be performed in walking people or under other natural conditions that are not easily accessible by other functional methods. Whether the detection of fast optical signals corresponding to neuronal activity will enable detection of signals in cases where the electrical or magnetic dipoles cannot be picked up on the head's surface remains to be elucidated in future studies. For developmental studies, it should be possible to examine young children who would not lie quietly in a magnet or a PET scanner. Interesting applications may also be found in clinical neuroscience. Children with attention deficits who are also not easily studied in PET or fMRI, may represent a group of patients accessible for optical studies. Monitoring cerebral oxygenation over several hours in patients at the bedside

is probably a prerequisite for the detection of PID in patients with stroke or SD equivalents during migraine attacks. The ability to detect PIDs could eventually lead to an application for optical methods in clinical neurology, since from recent animal data it seems plausible that pharmacological inhibition of PIDs is an efficient approach for stroke treatment[88]. Another potential clinical application is the detection of hematoma[90].

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