Methods 45 (2008) 289–299

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Non-invasive NIR spectroscopy of human brain function during exercise

Stéphane Perrey *

Faculty of Sport Sciences, EA 2991 Motor Efficiency and Deficiency Laboratory, University of Montpellier I, 700 Avenue du Pic Saint Loup, 34090 Montpellier, France

ARTICLE INFO

Article history:
Accepted 30 April 2008
Available online 6 June 2008

Keywords:
Neurovascular coupling
Haemodynamic response
Exercise
Monitoring
Brain physiology
Central fatigue
Technology
Near-infrared
Prefrontal cortex

ABSTRACT

The assessment of physiological changes associated with brain activity has become possible by optical methods, such as near-infrared spectroscopy (NIRS). NIRS is a useful neuroimaging technique based on haemodynamic principles for the non-invasive investigation of brain in motion. Due to its properties, the near-infrared light can penetrate biological tissue reasonably well to assess brain activity and two types of measurements are possible according to the number of channels used: dynamic changes in a localized brain region or functional brain imaging. The theoretical and technological advances of the past 10–15 years have opened the door to a range of applications in the human movement sciences, including some that involve imaging of the adult brain during motor and cognitive tasks, which for many years had been inaccessible to NIRS. This article examines the perturbation methods for measuring cerebral haemodynamic responses within resting and exercise conditions in humans and how NIRS can be used to image the moving brain. Methodological challenges of NIRS technique are presented, while the advantages and pitfalls of NIRS compared to other neuroimaging methods are discussed. Actual and future uses for NIRS in the field of sport sciences are outlined for a better understanding of brain processes during movement.

1. Introduction

The study of brain function progressed in the late 19th century through work involving the stimulation of the cortex of animal brains using electrical currents. This leads to the mapping of motor function in animals and, later, in humans. These first results however, contained many inconsistencies. In the latter half of the past century, most progress in the study of brain function has come from patients with neurological disorders. It has only been in the last two decades or so that brain neuroimaging techniques have allowed the study of healthy human subjects. With the development of the neuroimaging techniques of computerized tomography and magnetic resonance imaging it is possible to be more specific as to the location of brain regions involved in cognitive and motor functions. The measurement of the electrical signals over the scalp, arising from the synchronous firing of the neurons in response to a stimulus, known as electroencephalography (EEG), opened up new possibilities for studying brain function in exercising subjects. However, it was the advent of the functional imaging modalities of positron emission tomography (PET), single photon emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG) that led to a new area in the study of brain function. But, the experimental environments for these techniques are quite different from the daily ones, require strict motion restriction and may be found stressful for the subjects. Furthermore, the *in vivo* determination of the brain function in humans requires flexible, accessible and rapid monitoring techniques. So, to gain further insights into the brain function, a new neuroimaging technique completely non-invasive and with no strict motion restriction is thus desirable.

The first demonstration that near-infrared (NIR) light can be used to monitor the state of cortical tissues non-invasively through the skull was presented by Jöbsis in 1977 [1]. Because NIR light penetrates tissue several centimeters deep, NIR spectroscopy (NIRS) has become a quickly growing method to provide non-invasive monitoring for tissue oxygenation and haemodynamics of the brain. Using multiple wavelengths the concentration of constituents of tissue such as oxy-haemoglobin (HbO_2), deoxy-haemoglobin (HHb), water, lipids and cytochrome oxidase (CtOx) can be quantified. Several reports have now described the potential of the NIRS technique in measuring the haemodynamic changes related to the human brain activities such as motor [2] or cognitive [3] functions.

Continued technological advances have allowed designers of the NIRS systems to add multiple sources and detectors attached to the scalp, leading to increased coverage of areas of interest. At the same time, individual components have become smaller and more reliable. NIRS systems today often consist of little more than a probe with fibre optic sources and detectors, a piece of dedicated hardware no larger than a small suitcase and a laptop computer. So this technique is suitable for exercise conditions and offers good prospects on many neurovascular issues in the sport sciences to look at the brain when engaged during challenging tasks (motor and/or cognitive). It is well known that exercise challenges the cardiovascular, pulmonary and musculoskeletal systems and it can

* Fax: +33 0 4 67 41 57 08.
E-mail address: stephane.perrey@univ-montp1.fr

1046-2023/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymeth.2008.04.005

have profound metabolic effects. In contrast to our extensive knowledge about the peripheral adaptations to exercise, information about the effects of exercise on the brain function is rather limited. Brain activation is associated with surplus perfusion reflected in high values for NIRS-determined oxygenation. Noteworthy brain function deteriorates when its average oxygenation becomes reduced more than 10% whereas skeletal muscles maintain their activity despite O_2 saturation below 10% [4].

In view of the potential utility of NIRS for the study of brain activity during motor tasks, the present article is aimed at presenting the current status of human brain function based on the haemodynamic and metabolic principles visible with the NIRS technique. After an introduction to the theoretical and physical background principles underlying the NIRS method and equipments for the brain tissue measurement, the scope of this article covers a number of the aspects concerning the usual techniques and applications of NIRS used to image the moving brain in humans. In addition, the relevant physiological measurement modalities of NIRS are given, and advantages and limitations compared to other neuroimaging techniques are highlighted. Finally, the last section outlines possible uses for NIRS with respect to the activation of the prefrontal cortex (PFC) during challenging motor and/or cognitive tasks (i.e., attentional and fatigue processes during exercise).

The brain imaging techniques that are reported here all measure slightly different properties of the brain as it carries out cognitive and motor tasks. Because of this the techniques should be seen as complementary rather than competitive. All of them have the potential to reveal much about the function of the brain during cognitive and motor tasks. The more direct approaches for monitoring neuronal activities use EEG and MEG, which detect the electrical and magnetic signals induced by the neuronal firing across synapses. The indirect measurements, which include fMRI, PET, SPECT and NIRS, monitor the haemodynamic response that results from the increased oxygen consumption of the neuronal cells.

2. Background principles underlying the NIRS method and equipments

Jöbsis [1] was the first to note the differential spectral absorption of haemoglobin and the mitochondrial enzyme CtOx *in vivo* with NIR transillumination. Chance [5] introduced and reported the concept of differential spectroscopy in 1951, which provides the basis for modern *in vivo* optical techniques. During cortical activity, a neurovascular process occurs whereby changes occur in cerebral blood flow (CBF), volume (CBV), and metabolic rate of oxygen consumption ($CRMO_2$). This manifests itself principally as an increased demand for oxygen with the local vasculature responding through flooding the cortical area and surrounding tissue with oxygenated haemoglobin usually accompanied by a corresponding drop in HHb. Besides, the NIRS signal depends on the different optical properties of HbO_2 and HHb, and measures the blood volume and blood oxygenation regulation supporting the neural activity [6,7]. The brain activity thus is associated with changes in optical properties of the brain tissue.

2.1. Physical and mathematical principles

In order to discuss the technique of NIRS in more detail, it is important to establish an understanding of the manner in which the light propagates through brain. Propagation of light through biological tissue depends on reflection, scattering and absorption [1]. Reflection is mainly determined by the angle of the light beam in relation to the surface of the tissue, whereas scattering and absorption of the light within tissue are dependent on the wavelength. While scattering of the photons simply decreases with increasing wavelength, the absorption pattern is more complicated.

In NIRS technique, it is possible to measure changes in CBV and oxygenation associated with cortical activity through the use of light in the 650–950 nm wavelengths range yielding a cerebral haemodynamic monitor. The optical absorption and scattering properties of scalp, hair, skull and the meninges surrounding the brain allow photons of these wavelengths to penetrate into the surface of the cortex where they undergo scattering and absorption events within a wide range of chromophores in the tissue. The nerve cell bodies and their axons form the main components of the grey and white matter in the brain. Scattering in the brain is mostly attributable to the presence of lipids. The lipid content in the white matter is considerably greater than in the grey matter. This is largely due to the lipid-rich sheath that covers nerve axons in the white matter knows as myelin, which protects and electrically insulates the axons. Due to the high refractive indices of the lipids in the myelin, white matter has a significantly higher scattering coefficient than grey matter [8].

The Beer–Lambert law provides the physical and mathematical basis for NIRS. This law states that transmission of light passing through a solution of a colour compound (chromophore) is absorbed by the compound resulting in a reduction in the intensity of the emerging light. The relationship between the absorption and concentration of a chromophore provided by the Beer–Lambert law is:

$$A = \log(I_0/I) = \varepsilon c d \quad (1)$$

Where A is the absorption of light expressed as optical density (log of the ratio between intensities of incident and transmitted light I), c the chromophore concentration, ε its extinction coefficient, and d the thickness (optical pathlength) of the solution. The major difference between traditional transmission absorption spectroscopy and that of tissue spectroscopy is that photons do not travel in a straight line in a tissue. This is due in part because the photons must traverse several types of tissue and may be absorbed or reflected to varying degrees by the constituents of the tissue, which may change over time. The Beer–Lambert law is only valid under certain limited conditions: the light entering the medium must be monochromatic and perfectly collimated, and the medium itself must be purely and uniformly absorbing [9]. But one of the greatest obstacles to the application of the Lambert–Beer equation in a NIR monitor of tissue oxygen utilization still is the determination of pathlength. It is possible to ignore pathlength altogether, if actual concentrations are not sought. Instruments, such as pulse oximeters, which measure ratios of substances, do not require determination of pathlength. Instruments that measure absolute concentrations of substances do require this determination. NIRS instruments may be divided into two distinct types: concentration monitors and saturation monitors. The former may be further subdivided into those that only measure relative concentration changes and those that attempt to measure absolute concentrations. Relative concentration monitors do not require precise pathlength determination, whereas absolute concentration monitors do. Over the years, the problem of pathlength determination has been dealt with in increasingly sophisticated ways. But NIRS-derived data in the literature about cerebral oxygenation must be interpreted in the light of the method used to determine pathlength. Since photons do not travel directly from the source to a receiver, the effective optical pathlength (described as banana-shaped) is longer than the inter-optode distance (usually 40–60 mm for the brain). This results in the light traveling in a shallow arc with a penetration depth of approximately one half the separation distance, or 2–3 cm, into the tissue. The light is scattered in all directions, but is only picked up by the detectors in measurable amounts from the shallow arc. A modification of the Beer–Lambert law is required to begin to resolve these differences as follow:

$$A = \sum \varepsilon c d \mathrm{DPF} \quad \text{and} \quad G \tag{2}$$

In the Eq. (2) d is now the inter-optode distance, DPF is the differential pathlength factor [10] and G is a term to account for scattering losses. The DPF is a function how much scattering and absorption occurs and hence depends on the optical characteristics of the tissue [11,12]. The term differential pathlength factor comes from the fact that it enables the differences in absorber concentration to be derived from the differences in attenuation. The DPF is a multiplicative factor (>1) that expresses the actual pathlength for a given component, and the sum is over all the absorbing chromophores that contribute to the signal. Knowledge of the DPF is essential in order to carry out quantitative spectroscopy measurements but is less important for qualitative or relative measures. The most common means to gain knowledge of DPF with time is with time resolved spectroscopy (TRS that uses a picosecond light pulse) NIRS, which attempts to measure the time of flight of photons as they travel from the sending optode to the receiving optode [13,14]. A sensor detects the time necessary for penetration of the tissue and relates this to distance: the later a photon is detected, the greater the scattering and its pathlength. This measure then is converted to an effective pathlength and is in turn related to inter-optode spacing. Using this methodology results in DPF measurements range from 4 to 6.5 for brain, which means that the pathlength of photons is 4–6.5 times longer than the spacing between optodes.

However, subject-to-subject variability exists in the DPF of approximately 10–15%, which creates a similar uncertainty in the calculated concentration changes [15]. Further the extent of the contribution of each tissue to the total path is difficult to measure. For instance, in brain, the light traversing the skull passes through different tissues, skin, bone, dura mater, and cerebral tissue, each of which has different optical pathlength and hence to the DPF. Finally, the NIR instruments that use DPF and source-detector spacing to estimate pathlength monitor trends only. The absolute quantification is still not possible because the light loss caused by background tissue scattering and the measurement geometry is still unknown. To date the most sophisticated measure instruments can more accurately determine changes in the measured chromophore concentrations.

2.2. NIRS equipments

Even when the question of pathlength is resolved, there will remain some technical limitation to the applicability of existing instruments for trend monitoring or to assess concentrations. Several types of NIR spectrophotometry devices have been developed that vary in sophistication, ease of application, algorithms used and number of wavelengths employed [16] which confounds the ability to determine the physiologic validity of reported results. Increasing the number of measurement wavelengths enables more accurate estimations of changes in haemoglobin concentrations, with smaller contributions from other chromophores [17]. The lack of standardized software algorithms [13], wavelength selection, number of wavelengths, optode spacing and geometry among commercially available NIRS instrument may be partly responsible for the lack of widespread acceptance of the technology in the past and in some countries compared to fMRI.

In general, an NIRS instrument needs a minimum of three components before it can be used to measure the light attenuation in tissue. First, a light source that can provide a dynamic range of wavelengths ideally with a narrow bandwidth but also capable of emitting relatively high and adjustable power levels. A laser diode or light emitting diode is the most popular choice. To avoid any cross-talk effects, care should be taken when multiple discrete wavelengths are integrated within an optical fiber. Because the detected signals are often very small, increasing the source power will help to compensate for the "diffusion loss" in the tissue. The light detector forms the most critical component in the NIRS instrument. In theory, the light sensitivity will determine the limits on the changes in haemoglobin that can be detected. It is therefore essential in neuroimaging to have highly sensitive photodetectors to detect the low light levels remitted from the brain surface [9]. Last, some form of electronic component is needed to drive the light source but which also is capable of demultiplexing the original light signals from multiple measurement points to translate them to physiologically useful information. Since individual detectors can simultaneously pick up light coming from several locations and at multiple wavelengths, separating signals is necessary to avoid interference between neighboring light sources and also ensure that ambient noise is adequately identified. With the advances in semiconductor technology, it is possible to integrate all the driving circuitries onto a single chip and the size of the instrument is hence determined by the number of source-detector pairs and the display unit. A single-point spectroscopic measurement can be described as using an optode pair consisting of a light emitter and a detector. In localized cerebral NIRS the objective is usually to correlate changes in chromophore concentrations with the stimulation of particular regions of the brain. Maki et al. [18] describe NIRS studies in which motor activity (a finger movement stimulus) causes measurable, localized changes in haemodynamics of the adult human motor cortex.

By increasing naturally the number of source-detector pairs, the NIRS technique can be extended spatially to acquire multiple-spectroscopic measurements from the reflected light. The determination of spatial changes in tissue properties is increased by the development of the technique known as optical topography [19], which uses arrays of multiple NIR sources and detectors arranged over the scalp surface. With a sufficient number of sources and detectors placed around the head it is feasible with sophisticated algorithms to generate cross-sectional or three-dimensional images of the optical properties of the brain. By the use of two or more optodes, regional information may be compared to distinguish between general, systemic responses to stimuli (e.g., due to oscillation in arterial blood pressure during dynamic movement) from regional responses due, e.g., to focal neuronal activation. Neuronal activation is associated with a rapid vasodilatation to meet the increased metabolic needs and oxygen uptake with increased oxygen supply. In fact, the vasodilatation precedes and overshoots the need. As a result, [HbO_2] increases (detectable by NIRS) and [HHb] decreases (detectable by NIRS or the blood-oxygenation-level-dependent (BOLD) effect using fMRI).

For the quantification of functional activity of the brain different NIRS methods are available. The conventional non-spatially resolved continuous wave (CW) NIRS only measures changes (D) of HbO_2 and HHb from an initial value by assuming a constant tissue scattering effect, thus serves only as a trend monitor. This is the type of measurement made in the earliest NIRS studies to study the haemodynamics and oxygenation of the human brain. With CW NIRS, the sensor placed on the scalp picks up a continuous stream of light from the light source, as opposed to time-resolved or phase-modulated forms of NIRS in which periodic samples are taken at the site [20,21]. The light in the range of 650–950 nm is used because (a) these wavelengths show good penetration of biological tissues, (b) the heme groups of haemoglobin are among the primary absorbing compounds, and (c) the absorption of light by the heme groups is altered by oxygen [20]. Most CW NIRS devices uses white light with specific filters on the detectors [20,22], although some employ unfiltered light detectors, and specific wavelength light sources [20]. The CW-type instruments allow observing dynamic changes in regional CBV in real time by measuring the concentration changes in total haemoglobin (Hbtot). The CW method only allows the continuous quantification of

relative values and usually relies on the DPF method. However, the attachment method and pressured applied of the optodes on the scalp may contribute to errors when assuming a fixed DPF. Another disadvantage is the relative sensitivity of the CW NIRS instrument to possible motion artifacts. This is particularly problematic during some exercise such as during running. The advantages are that the CW NIRS instrument is inexpensive and can be miniaturized to the extent of a wireless instrument. And by using many source-detector pairs, the CW-type instruments can be used for imaging the brain.

In order to quantify haemoglobin concentration changes the TRS instrument, also known as time domain spectroscopy has been established as a laboratory-based device but appears difficult to use in an open field. The TRS instrument requires an ultrashort pulsed laser and a detection system that can detect the emerging intensity as a function of time with picosecond resolution. Due to the scattering process, the pulse will broaden and, due to absorption will be reduced. The TRS method is able to yield a lot of information (e.g., absolute values of chromophores concentration) relatively rapidly and with a high dynamic range. Because this instrument operates in photon counting mode, it is highly sensitive and can penetrate relatively large tissues as the head. However, due to the low number of photons, a relatively high level of noise characterizes TRS measurements. Further this type of instrumentation is so far of a big size. The need for several wavelengths of the picosecond light source and a fast detector makes this an expensive technique.

One of the most NIRS technique used is the NIR spatially resolved spectroscopy (SRS). The SRS system incorporates several detectors housed in a single probe, which is placed 4–5 cm from the source for the head. The combination of the multi-distance measurements of optical attenuation allows calculation of the relative concentrations of HbO_2 and HHb in the illuminated tissue. This calculation is derived from the relative absorption coefficients obtained from the slope of light attenuation at different wavelengths over a distance measured at several focal points from the light emission. The SRS–O_2 value represents an estimate of mean tissue oxygen saturation equivalent to the arterio-venous O_2 saturation determined by a blood gas analyser. The difference between these two parameters is that the arterial and venous O_2 saturations reflect only their respective vascular compartments, while the SRS–O_2 saturation reflects predominantly the mean of arteriolar, capillary and venular O_2 saturations. In addition, arterio-venous O_2 saturation indicates differences across the brain, whereas the SRS value measures a discrete region of the brain. NIRS in adults has been hampered by concerns over contamination from extracerebral tissues. By using a SRS approach, the superficial layers of brain tissue affect all the light bundles similarly and therefore their influences cancel out. Only deeper tissue layers have an effect on the values [23]. SRS is technically simpler than TRS and provides measurements with a good signal-to-noise ratio and a high time resolution.

2.3. Usual methods for brain activity measurements by NIRS in humans

Consider now the assessment of brain oxygenation by SRS NIRS using a single (Fig. 1) or two-channel tissue oximeter. After the probe test in a phantom calibration box to analyse the total probe sensitivity and the sensitivity difference between sensors of the detection probe, one or two sets of emission and detection probes have to be bilaterally attached to the forehead of the subject by employing the landmarks of the international 10–20 system for EEG electrode placement [24]. Prefrontal cortex (PFC) is known to project to pre-motor areas and to be responsible for movement planning and pacing strategies, as well as decision-making [25,26]. It was recently shown that decreased frontal cortical oxygenation was associated with reduced muscle force-generating capacity [27]. According to the protocols and tasks, the optical probe comprising one emitter and one detector may be positioned differently. For example, the light emitter is placed at the EEG position Fp1 for the left side (at Fp2 for the right side) and the light detector is placed between F7 and F3 on the left (between F4 and F8 on the right). With this setup, NIRS measures oxygenation between these two points, corresponding to Brodman Area 10 and Brodman Area 46 in the PFC. Probes have to be placed high on the ipsilateral forehead to avoid the temporalis muscle and sufficiently lateral from the midline to avoid the superior sagittal sinus. In each set, the

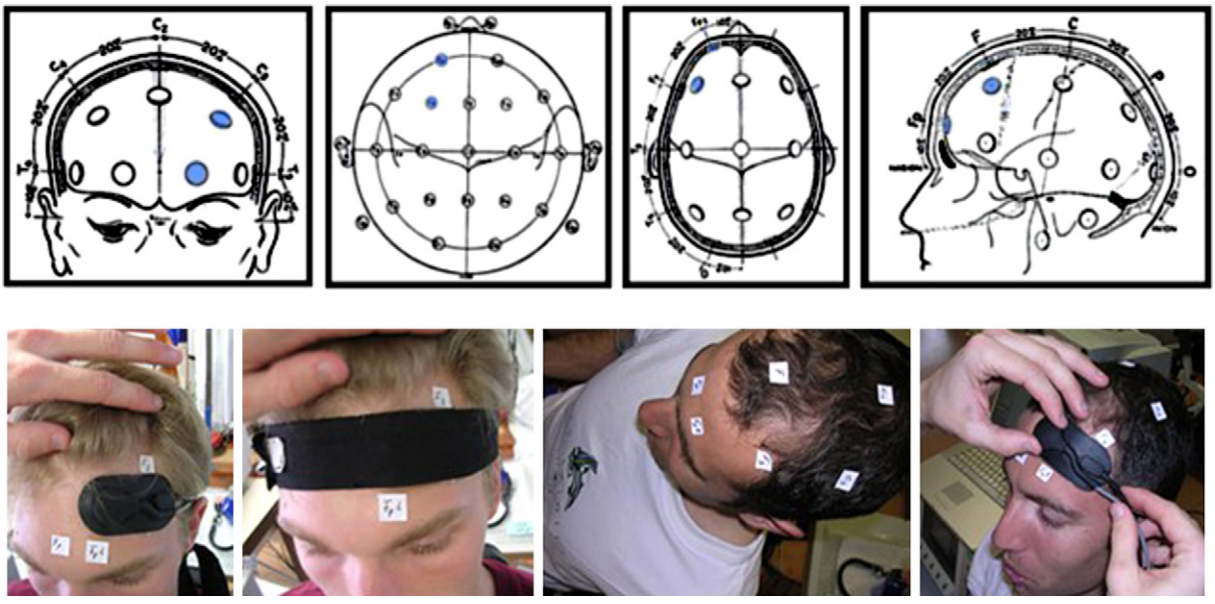

Fig. 1. To assess brain oxygenation with NIRS, the detection probes are positioned over the left prefrontal cortical area between Fp1 and F3, according to the landmarks of the international 10–20 system for EEG electrode placement. The inter-optode distance is kept constant at 5 cm and the probe holder is covered and maintained with a homemade black Velcro headband.

emission and detection probes should keep a constant geometry and distance of 5 cm in a specialized rubber holder and secured to the skin with a double-sided adhesive sheet. In addition, the optode holder has to be secured still further by a crepe bandage around the head to prevent sliding during data collection. Adjustments then are necessary. The probe positioned around the motor area for the working limb is checked by a simulated motor task to induce functional oxygenation. If no oxygenation changes are detected in response to the simulated task, the probes are moved by several millimeters by trial and error until a consistent oxygenation response is achieved. No sliding of the optical probes should be observed throughout the data collection. The optode positioning is important to assure appropriate penetration of photons to deep structures, avoid stray and short path light and prevent recording primarily from more superficial structures. These goals are most easily achieved using a reflectance mode wherein the source and detector fiber optic bundles are spaced several centimeters apart on the skin overlying the tissue. Dark clothes may be used to cover the area of interest in order to avoid surrounding light and to absorb superficially traveling photons, thereby suppressing the influence from skin. Minor changes in optode-skin contact induce large transients in the signal and/or baseline shifts. Constant optode distance also is crucial; if the head circumference changes even by a fraction of a millimeter as a result of change in brain blood or brain water content, the trends are significantly biased. After fixing carefully the probe holder(s) on the subject, an initialization procedure is often carried out according to the NIRS devices. This procedure allows setting of each laser power, automatically establishing the optimum measurement condition. The NIRS data then may be recorded with a relatively high sampling rate (1 to more than 100 Hz) depending on the NIRS instrument used.

Several types of brain activity have been assessed by NIRS [6] by using single or two-channel instruments. Recent studies have been carried out with multichannel NIRS instrument (e.g., 24 channels and high temporal resolution less than 1 s [28]). The probes of the NIRS instrument are fixed using rubber shells on the subject's left and right frontal areas. The shells are covered with a nylon net to keep them attached to the head. Each shell is capable of measuring the relative concentrations of haemoglobin at 24 points in a 9×9 cm^2 area. This recorded area corresponds to the bilateral superior frontal gyrus and middle frontal gyrus (Brodman Areas 6, 8, 9, 10). The lowest probes are positioned along the Fp1–Fp2 line in accordance with the international 10/20 system used for EEG [24]. The center of the parietal probe is located at P3/P4, and the occipital probe is on the O1–O2 line. The correspondence of the probe positions then is usually confirmed by superimposition of the probes on a representative three-dimensionally constructed MRI of a healthy volunteer.

3. Approaches for quantitative NIRS measurements of brain activity

Brain activity monitoring is not new. Many techniques are available to monitor different aspects of brain activity: EEG, transcranial Doppler, brain saturation via jugular bulb oximetry or PFC NIRS. NIRS is promising in that the contrast mechanism for the signals is closely related to that of intrinsic optical imaging of exposed cortex using visible light. NIRS technique utilizes changes in blood volume and haemoglobin oxygenation (i.e., haemodynamics as an index of neural activation [6,7,29]). The rationale for this approach rests on the concept that neural activation in response to a stimulus results in increased energy demands in the area activated. To accommodate the demand for energy, CBF increases to the activated brain.

How does it work? When a specific area of the brain becomes metabolically active, each of the individual nerve cells produces an electrical signal, which is transmitted along the axons and dendrites to the adjacent cell membranes. Each of these action potential signals causes an increase in oxygen consumption by the neuronal cells and induces a secondary increase in CBF and CBV to meet the increased demand for glucose and oxygen. The increase in regional CBF and $CMRO_2$ is a consequence of regional brain activity. As haemoglobin is the major oxygen carrier in the blood vessels, this causes an increase in HbO_2 accompanied by a decrease in HHb (Fig. 2) during functional activation [2,18]. Evidence that there is a linear relationship between haemodynamics and neural activity [30] and that NIRS produces results consistent with other imaging techniques (fMRI and PET) used simulta-

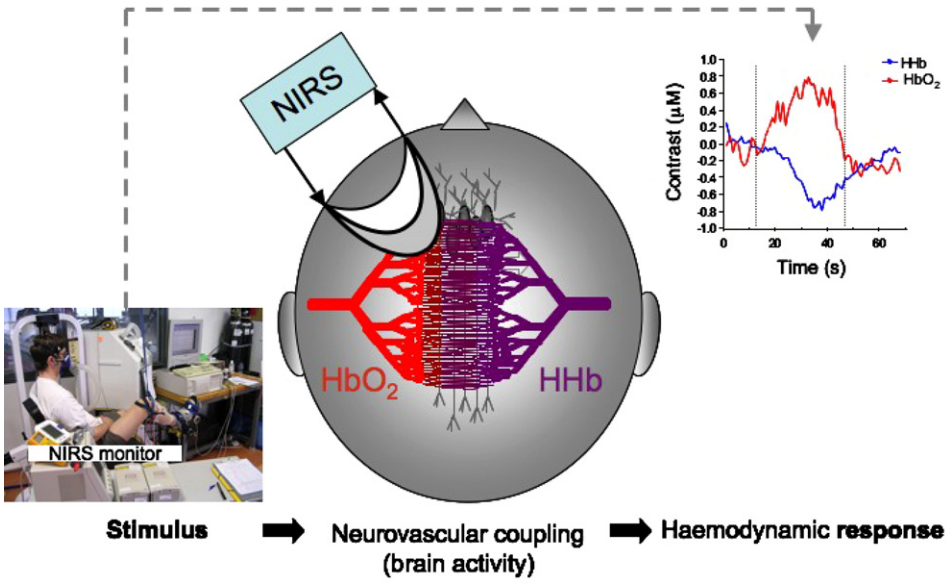

Fig. 2. The oxygenation response over an activated area of the cortex in response to a sustained isometric contraction of the ankle extensors at a moderate intensity for about 30 s can be described by a decrease in deoxy-haemoglobin (HHb) along with a simultaneous increase in oxy-haemoglobin (HbO_2). Near-infrared light (assumed banana-shaped light path) illuminates the brain through the skin, scalp and skull, and is diffusely reflected back towards the detectors. HHb and HbO_2 are mainly of interest because they are related to the regional cerebral blood flow (CBF) changes. The focal change in regional CBF determines the activation state. See text for further details.

neously [31,32] provides converging evidence that NIRS can provide a reliable measure of brain function.

Despite of the limited ability to measure the deep tissues (see below), NIRS can be used as a continuous monitor of changes in cerebral oxygenation and blood volume by following on-line trending of changes in the concentration of HbO_2, HHb and hence Hbtot, which is proportional to changes in CBV, which in turn can be used as a surrogate measure of CBF. The variety of data that can be measured by NIRS (real-time cerebral oxygenation and CBV changes, and absolute CBV and CBF measurements) contributes to its potential in the field of sport sciences. In order to determine blood volume or flow one has to induce a perturbation in the brain tissue to produce a change in the baseline level of the total haemoglobin concentration. Such experimental perturbations are jugular occlusion, inhalation of different fraction of inspired oxygen (FIO_2) and head tilting.

3.1. Measurement of cerebral haemodynamics and oxygenation

Traditional methods of examining CBF and CBV tend to be rather invasive. In contrast, NIRS offers the potential for non-invasive monitoring of changes in CBV and CBF.

The continuous measurement of changes in Hbtot concentration generally reflects changes in CBV. Absolute quantification of CBV is possible with NIRS. If arterial saturation is reduced by a small increment (approximately 5%) in a controlled manner (allowing full equilibrium within the cerebral microcirculation) HbO_2 is observed by NIRS to decline in parallel. If arterial saturation is plotted against HbO_2 concentration, the gradient of the resulting straight line is directly proportional to CBV. This measurement can be used to provide a baseline value for CBV with which the real-time trends in Hbtot concentration can be compared. Changes in CBV in humans have been validated against strain gauge plethysmography. However, the use of NIRS method may underestimate CBF and this is likely to relate to contribution of non-cerebral in the field of view. The head is not opaque to infrared light, but scatters the light very intensively; computer modeling [33] has shown that in a typical volume of tissue interrogated by NIRS, about 30% is brain and 70% is non-cerebral (i.e., scalp and skull). This means that is necessary to make several measurements in order to obtain a reliable value for CBF. Nevertheless, the non-invasive nature of NIRS compared with other methods of measuring CBF is a huge advantage, which may offset the greater variability of a single measurement.

The measurement of CBF by CW NIRS is based on the Fick's principle and uses a rapid change in arterial oxy-haemoglobin as an intravascular tracer. The rate of accumulation of a tracer in a given tissue is equal to its rate of inflow minus its rate of outflow. When the tracer is introduced rapidly and its rate of accumulation is measured over time, blood flow can be measured as a ratio of the tracer accumulated to the quantity of tracer introduced over a given time. So, a small reduction in arterial saturation (about 5–10%) is induced by lowering FIO_2. When a stable baseline is achieved, a breath of 100% oxygen creates a bolus of HbO_2 in the arterial circulation, which acts as the required Fick tracer. The arrival of HbO_2 in the brain a few seconds later can be observed by NIRS. CBF may be calculated by considering the ratio of the rate of HbO_2 accumulation in the brain to the amount of oxygen delivered. The method of measuring CBF rests on several assumptions: first, blood flow must be constant for the period of measurement; second, there must be linearity and stability of the tracer dose response in tissue; third, the tracer must not be metabolized or permanently retained in the tissue during the period of measurement and fourth, the period of measurement must be less than the cerebral transit time (approximately 10 s). Measurements of blood flow with NIRS have been compared favorably to ^{133}Xenon clearance, a well-established technique for measuring CBF. These comparisons constitute important direct external validation of NIRS in the human brain; the agreement between the two methods is acceptable [34].

Jugular venous bulb oxygen saturation is invasive and prone to artifact, even in experienced hands. Cerebral venous saturation may be estimated by NIRS. It has been assumed that change in CBV, induced by jugular occlusion or head tilting, is due to a change in the quantity of blood in the venous sinuses. Therefore, the relative change in HHb compared to HbO_2 can be used to calculate the saturation of cerebral venous blood ($CSvO_2$).

The non-invasive method of measuring $CSvO_2$ with partial jugular venous occlusion was validated with an invasive measurement of SvO_2 from co-oximetry of jugular bulb blood obtained during cardiac catheterization and gave similar values [35].

To estimate $CSvO_2$ in absolute terms without impeding venous outflow, the SRS NIRS equipment allows easy monitoring of the ratio of absolute HbO_2 to Hbtot (i.e., called cerebral tissue oxygenation index or TOI for haemoglobin saturation). TOI applied to the head is a surrogate measure for $CSvO_2$. Typical values are 60–80%. The signal noise is usually 2–3%. If averaging over a minute is done, a very precise mean value may be obtained.

3.2. Validity of cerebral NIRS measurements

Non-invasive characterization of blood flow and metabolism in brain has important applications in the sport sciences. Improved measurements of these quantities may lead to improve fundamental understanding of the brain function during prolonged exercise. Invasive techniques provide the most direct assessment of blood flow and oxygen delivery and have been used to assess the validity of non-invasive technologies such as NIRS.

Quantifying oxygen delivery to tissue is methodologically difficult. Methods to assess blood flow and oxygen utilization have utilized venous plethysmography and the Xenon technique. However, all these methods are difficult to apply to the brain during exercise. There are three main categories of methods able to assess blood flow in tissues: anatomical (nuclear MRI, PET); direct (plethysmography, ^{133}Xenon inhalation, laser Doppler flowmetry and Doppler ultrasound); indirect (nuclear magnetic resonance spectroscopy, NIRS). Each of these techniques has some disadvantages such as limitation given by tissue (plethysmography), is invasive (^{133}Xenon inhalation), and uses expensive equipment (nuclear magnetic resonance imaging, PET, laser Doppler flowmetry), or assesses superficial blood flow (laser Doppler flowmetry).

Some non-invasive techniques provide global measures of blood flow, with Doppler imaging corresponding to the volume of flow in a large artery, and plethysmography measuring changes in volume corresponding to total arterial inflow [20]. Although useful, these methods do not provide a measure of oxygen delivery and utilization in tissue. Conventional venous occlusion plethysmography does not provide regional information and can be used only in a static state or during brief period of exercise, since venous occlusion interrupts blood flow. Ultrasound Doppler is a common tool used to measure blood flow in large vessels. However, it is not sensitive to flow in smaller vessels, and does not readily permit continuous measurements during exercise. Laser Doppler can non-invasively monitor flow changes, but most systems measures the tissue surface only or relatively deeper tissues using larger source-detector separations [36]. In this context, one of the strongest points of NIRS is the reduced motion restriction of subjects. The newly developed portable, telemetric NIRS system allows subjects to freely move about even during measurement [37].

4. Comparison with alternative neuroimaging methods and usability of NIRS method

The oldest method for eavesdropping on the brain is termed EEG. The EEG technique measures fluctuations in electrical activity over time with an excellent temporal resolution. It also has an advantage that many researchers find appealing: it is inexpensive. Many later developments, such as event-related potential and MEG, are variations on EEG. PET is perhaps the most interesting. To perform a PET scan, a radioactive isotope is injected into the blood of the individual. Considered invasive and expensive, this method measures how much blood is detected in the brain when the latter is active. As blood flow increases to different areas, the machine picks up the radioactive elements introduced into the blood. These measurements are run through a computer program that constructs a three-dimensional image. A well-known technique for studying various parts of the body, magnetic resonance imaging, or MRI, was originally designed to assess structural, rather than functional, components. This method is expensive but non-invasive. Based on structural MRI, BOLD fMRI is a method of observing which areas of the brain are active at any given time. The most current brain observation technique is NIRS that combines the strengths of fMRI and EEG to produce excellent spatial and temporal data regarding processing in cortical tissue.

The research into NIR based brain activity monitoring was motivated by its potential as an alternative to older and more established imaging modalities such as fMRI and PET. There are several motivating factors for researching the potential of NIR based methods. For one, the NIR method provides information about physiological parameters not available in other modalities, such as oxygenation information. Secondly, NIR equipment has better signal-to-noise ratio compared to fMRI and PET [32]. This allows for, among other things, being able to model fast oscillatory noise related to normal physiological functions. Thirdly, NIR equipment is relatively less restraining compared to fMRI or PET and generally safer than PET as it does not rely on ionizing radiation. Some types of NIR equipment, namely those using the CW signals, also has been made portable and in some instances, telemetric.

An obvious question when considering NIRS as a method for assessing brain function is what advantages it offers over other techniques? The use of NIRS has several distinct advantages over other, more traditional brain-imaging techniques. In contrast to other techniques, in particular fMRI, one advantage is that NIRS offers biochemical specificity by measuring concentrations of biochemically well-defined substances such as HbO_2, HHb and CtOx. An intrinsic advantage of NIRS over fMRI BOLD is that the latter provides only a measure of HbO_2 whereas the former can use two (or more) NIR wavelengths to provide separate measures of HbO_2 and HHb. The two measures are potentially advantageous in separating signals due to increased flow from signals because of increased oxygen consumption (an important issue in studies with fMRI BOLD). A second advantage is that, NIRS has good temporal accompanied with a limited spatial resolution to a lobar level. Brain signals can be routinely observed [38] with a temporal sampling resolution of 0.01 s, which is faster than that typically observed with fMRI. Even if the haemodynamic response to the brain activation occurs on a 1 s time scale, the better temporal resolution offered by NIRS will, for instance, enable better distinction of signal contamination arising from systemic physiological signals and motion artifacts, better resolution of the haemodynamic onset, and potentially, enable direct measures of fast neuronal signals. In addition, the effects are localized within 1–2 cm of the area activated [39]. Compared to electrophysiological techniques (EEG, MEG) where source localization is difficult, spatial resolution appears quite good. A third advantage is that NIRS is totally non-invasive and non-ionizing. Hence, it is safe to use with humans repeatedly and for extended periods of time. A fourth advantage is that it is relatively inexpensive, portable, and with the appropriate training, relatively straightforward to use. This makes NIRS particularly attractive to researchers in the experimental setting.

One potential disadvantage of using NIRS is that, because near-IR light diffuses rapidly when entering neural tissue, it is unsuitable for investigating neural activation in structures deeper than approximately 2–3 cm below the surface of the brain. The depth of penetration is dependent on optode separation, and scatter tends to be random and unpredictable. In addition, the cerebrospinal fluid layer, by causing a uniform distribution of NIR light, may alter the calculated spatially resolved spectroscopy slope (SRS method) and leads to abnormally low values for saturation. The skull itself and its interface with other layers in the human head may distort the penetration of NIR light by generating an optical channel. However, if the neural structures of interest fall on or near the surface of the cortex, as they do in some researches in sport sciences, NIRS is an ideal neuroimaging technique. Thus, compared to other functional neuroimaging methods such as PET and fMRI, NIRS lacks spatial resolution (even if better than EEG) 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 (i.e., haemodynamics changes measured from the scalp include systemic vascular effects not only from inside but also from outside the brain). Consequently it is important to use experimental tasks that do not evoke any large systemic vascular changes, unless they are monitored independently (muscle blood flow, cardiac output, oxygen saturation). These non-neural vascular responses may contaminate the NIRS signal but their measurements are pretty useful in reducing them from the signal of interest.

The usability of NIRS methodology also is dependent on several factors like the equipment itself, the optode positioning and geometry and the inter-optode distance, the number of wavelengths and the nature of the algorithm used for calculation of the results. Most biophysical investigations of NIRS indicates that a minimum of four wavelengths is necessary to generate algorithms for NIRS, and position of the probes has to be as close as possible to the location of change, which is typically achieved by selecting a recording location to maximize signal. Like EEG, NIRS requires attachment of a set of probes (NIR emitters and detectors) on the scalp, but in contrast to fMRI, it does not expose humans to high magnetic fields. Although NIRS does not require the strict head-motion constraint of MRI, any slippage of the probes on the scalp, or variations in the intensity of the NIR light at the point of contact with the scalp, will lead to spurious signals at one or more detectors. A number of laboratories has suggested some solutions to these movement artifacts especially during exercise. First, solution is to design probes and probe-holders that reduce gross movement artifacts during exercise such as running, walking compared to cycling and rowing. Second, statistical techniques (cross-correlation, principle components analysis or PCA) to eliminate signals with shared variance have to be used. Third, solution is to define automatic algorithms that reject signals that fall outside the range of haemodynamic responses due to neural activation (e.g., signals arising from the cardiac and respiratory cycle). After optical topography recording, data reduction is often realized as follow: the amplitude of changes in HbO_2 waveform in each area during the experimental task is expressed as the activation index. The index is calculated by the signal processing of (i) excluding visually the motion artifacts (spikes), (ii) averaging the data [18], (iii) PCA which removes much of the co-variance due to motion (i.e., subtract the non-neural signals and leave the residuals, [40]) of the averaged [HbO_2] data in a probe to obtain the representative time-course, and (iv) fitting the

representative time-course calculated by PCA to a haemodynamic response.

5. Laboratory and field applications with NIRS to image the exercising brain

Based on the current status of the developments in optical imaging and considering relative advantages and disadvantages as compared to other functional neuroimaging methods, applications in sport sciences are appealing for NIRS. As with any new technology and method, initial research using NIRS in sports sciences has been primarily descriptive in nature. NIRS has been used extensively in an attempt to monitor the state of cerebral oxygenation trend in healthy subjects, during and after exercise. Neuroimaging techniques have advanced to a point where it is possible to track regional CBF with a spatial resolution of some millimeters, such as PET, fMRI and SPECT. Although of these techniques can be applied to exercise with low intensity [41] there remains difficulties in using the imaging techniques during exercise that involves large muscle groups. PET and fMRI require that the head of the subject remains motionless, which may be impossible during intense exercise [41]. Furthermore, monitoring cerebral oxygenation with NIRS for prolonged exercise may be useful in localizing physiological brain activity, particularly when other methods are not applicable.

5.1. NIRS signals and brain activity during movement

The brain may be challenged to accelerate its metabolism during activation in responses to a motor or a mental/cognitive tasks involved in exercise. Dynamic movement is associated with cortical activation and increases in blood flow to the supplementary motor area and the primary sensorimotor area [42]. Such regional flow changes (CBF) are accompanied by a much smaller increase in regional metabolism ($CMRO_2$), which results in a decrease in HHb in venous blood. Thus, a decrease in HHb accompanied by an increase in HbO_2 of 2- to 3-fold magnitude, resulting in an increase in Hbtot, is expected to be observed in activated cortical areas in NIRS measurement. However, small changes in CBF are not always accompanied by those in Hbtot and HHb [43]. Thus, when cortical activation is defined based on NIRS measurement, HbO_2 is the most valid parameter. The term activation has been operationally defined by the focal increase in regional CBF for all methods based on the vascular responses to a stimulus. By analogy, a decrease in regional CBF is termed deactivation. Recent imaging studies have shown that alternating leg movements, such as occur in walking, running, and bicycling (Fig. 3A) activates PFC, the primary motor region and the supplementary motor region. Fukuyama et al. [44] showed, using SPECT, that the primary sensorimotor area and the supplementary motor area were activated with back and forth walking along a 20 m long corridor. Miyai et al. [45] showed, by an optical imaging technique using NIRS, that the concentration of the HbO_2 was bilaterally increased in the medial primary sensorimotor region and the supplementary motor region during walking at 1 km/h on a treadmill. Christensen et al. [41] showed, by PET, that during active bicycling the primary motor cortex, the primary sensory cortex, and the supplementary motor cortex are bilaterally activated. Further, a PET study showed that imagining an exercise increased the activity of the right superiodorsal sensorimotor area [46]. Finally, a gait imaging or passive performance of bicycling study showed activation of the primary sensory cortex, the primary motor cortex, and the supplementary motor cortex [41,45]. Ide et al. [47] showed, by NIRS, that the concentrations of HbO_2, HHb and Hbtot in the prefrontal region increased during bicycling at a submaximal work intensity. Thornton et al. [46] showed that imagining freewheel bicycling activated the right dorsolateral-prefrontal cortex and the right premotor cortex. And Suzuki et al. [26] showed, by an NIRS technique, that during a 9 km/h running on a treadmill, the HbO_2 of the prefrontal region was increased. Although NIRS is not as sensitive to motion artifacts as fMRI and PET, any quick motion shaking the optical fibers may cause sharp changes in haemoglobin signals. Therefore, it should be instructed subjects to keep their heads stiff during measurements. Improvements to the experimental design as well as the fiber holder also are essential to solve these problems.

5.2. Cerebral oxygenation changes in response to fatigue

During exhaustive and/or prolonged exercise, subjects may exhibit a decrease in cognitive and physical abilities. Such manifestations could represent a reduction of cerebral blood flow and oxygenation. NIRS is able to monitor changes in cerebral oxygenation during dynamic prolonged exercise. Due to its applicability to any local tissue, NIRS also can be an excellent tool in understanding the phenomenon of fatigue [48–52]. Muscle fatigue results from not only peripheral fatigue but also central neural system [53] and decrease of cerebral function during exercise has been reported [48,53]. Over the past 3–5 years there has been a flurry of research in this area and has included the measurement of muscle and cerebral oxygenation during maximal exercise [48–51]. What is often overseen is that the increased motor command leads to increased metabolic rates in the activated brain structures associated with a given physical activity, which are commensurate with exercise intensity. Previous studies, which monitored cortical activation during exercise using NIRS, reported that cerebral oxygenation during submaximal, maximal, and supramaximal cycling exercise increased from the resting values [26,48–50]. With NIRS, it is possible to determine PFC oxygenation during sustained static and dynamic exercise to voluntary exhaustion (Fig. 3) and oxygenation profiles until the exact moment of voluntary exhaustion. The increase in cerebral oxygenation during activation by exercise is in contrast to the progressive reduction in muscle oxygenation with workload (Fig. 3B). At high exercise intensity and hence intense regional neuronal activity, energy demand may exceed energy supply, and an imbalance may occur in brain regions activated during exhaustive exercise. Recent research has shown that fatigue is preceded by reduction in cerebral oxygenation [48,49,51]. In these studies, findings indicated a decrease in central oxygenation before motor performance failure during exhaustive exercise, which may be compatible with the notion of a role for the cortex in the reduction of motor output at the cessation of exercise. Under these experimental conditions, NIRS shows a rise in HHb and a drop in HbO_2 (Fig. 3B), hence the reverse behavior as seen in cerebral activation. Such a deactivation phenomena in the PFC during performance of mental tasks also has been described in a simultaneous PET–NIRS study [54]. It is also fascinating how the brain, for hours, can stress the body during a marathon run for example. Prolonged exercise causes a marked perturbation of cerebral metabolism [55] and the subsequent breakdown of running style suggests that the muscles are no longer activated ideally. Ross et al. [56] provided recent insights into the phenomena within the central nervous system that are affected by long-lasting exercise. The actual applicable methods (neuroimaging techniques as NIRS and transcranial magnetic stimulation) would benefit to be combined in order to elucidate better how, when and why central fatigue develops during exercise. What emerges is that strenuous and prolonged exercise is a challenge to the oxygenation of the brain. In that regard, changes in CBF for tracking brain activation by cerebral NIRS-determined oxygenation can be useful at least quantitatively.

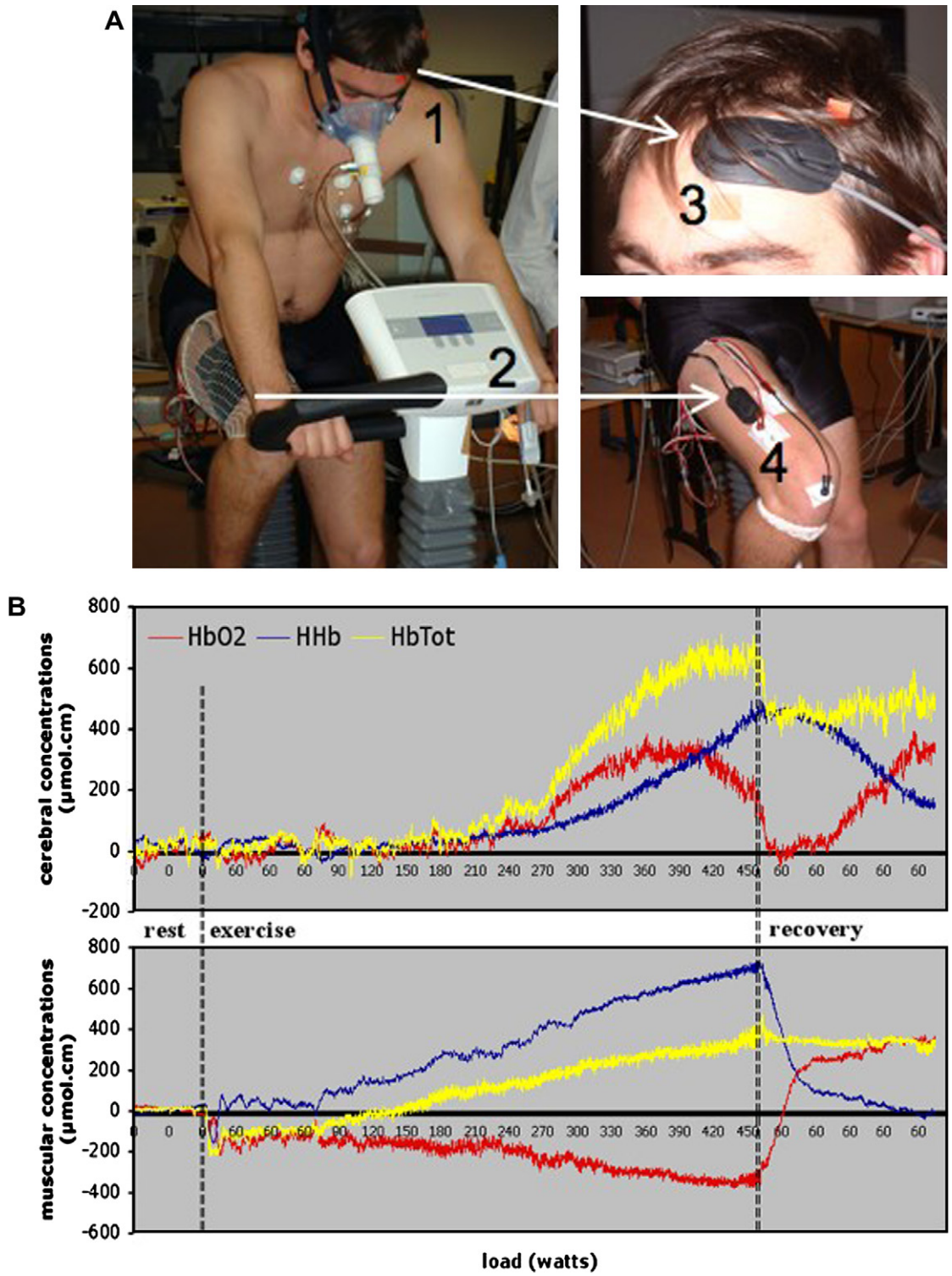

Fig. 3. (A) Experimental set-up (1: gas exchange metabolic system, 2: cycling ergometer, 3: NIRS optodes on the prefrontal cortex region, 4: NIRS optodes on the vastus lateralis muscle). (B) Simultaneous time-course changes (right) in concentration of cerebral (top) and muscular (bottom) oxy-haemoglobin ([HbO_2]), deoxy-haemoglobin ([HHb]) and total haemoglobin ([Hbtot]) caused by a progressive maximal cycling exercise. Values are presented for a representative subject. Modified from [48].

5.3. Perspectives on exercising brain and cognition

Exercise involves providing the subject with goal-directed tasks, and most exercise also requires subjects to pay attention to external stimuli continuously. Neuroimaging studies have shown a significant relationship between cognitive function and PFC activity [57]. Although NIRS has, compared with fMRI, lower spatial resolution and is limited to determination of activity over the surface of the brain, NIRS is tolerant of motion artifacts, and allows investigation of diverse neurocognitive processes associated or not with motor tasks. In competitive athletic races of walking and running, a phase to prepare for movements is traditionally set, forcing athletes to take a specific posture. PFC plays a crucial role in preparation of forthcoming actions and may serve as the supervisory

system to sustain attention for exercise [58]. Also, some studies support the hypothesis that fatigue affects high-level cognitive processes [59]. Future research will have to address a number of issues related to the finding that prolonged exercise selectively and transiently impairs prefrontal-dependent psychological and physiological processes. Measures with NIRS combined with other selective neuropsychological measures are needed to further explore the complex interaction between exercise and mental function. NIRS monitoring throughout exercise on PFC for an individual's ability to make cognitive judgments, while operating under physical constraints is possible. Changes in haemodynamic responses probably will arise from attention demand (or vigilance) for visual stimuli for instance.

For tasks requiring substantial bodily motion, the brain has to make due with a finite amount of resources. This builds on the fundamental principle that processing in the brain is competitive [25]. Because sensory-motor integration tasks require massive and sustained activation of sensory, motor, and autonomic systems, an individual may need to inhibit neural activity in regions performing functions that the individual can afford to disengage. These regions are, first and foremost, the higher cognitive centers of the PFC (see [60,61]). As hypothesized by Dietrich and Sparing [61] prolonged exercise might result in a state of transient "hypofrontality". Endurance exercise selectively compromised prefrontal-dependent executive functions such as sustained attention. The HbO_2 decrease in PFC probably reflects neural inhibition, which is derived from attention demand for exercise. In this respect and according to recent studies demonstrating changes in cortical activation after fatiguing protocols [62,63], important insight would be gained from the observation of some attentional and motor tasks (e.g., in a double task design with NIRS data collection: bimanual coordination task performance on long time scales or prolonged cycling with cognitive tasks such as mountain bike or other outdoors activities as hiking poles where visual stimuli, that is attentional control, is high) as well as from the exploration of relationship between neural activity, neuromuscular and behavioral dynamics. Given current views of HbO_2 from NIRS as a neurovascular index of higher brain function processes triggered by the attentional demand and/or task requirement, the analysis of the HbO_2 signal in motion provides a test of the relative contribution of brain activity monitoring processes during task execution for individuals. Recent advances in physiology, psychology and neuroscience have greatly enhanced our understanding of the contribution of the PFC to exercise. In the future, this will allow exercise scientists to ask more specific questions regarding the effects of physical exercise on cognition using a promising NIRS methodology that makes use of this knowledge.

6. Concluding remarks

The NIRS technique proved to be an effective and independent source of neuroimaging method. NIRS is a powerful tool for investigating functional activation of the human cerebral cortex by tracking changes in cerebral O_2 status across a wide physiological relevant range from rest to intense physical work. To a degree, NIRS offers advantages where other neuroimaging methods such as fMRI, PET, and EEG are lacking. In addition, NIRS provides excellent temporal resolution and produces good spatial resolution (1–2 cm), affordability, and is safe for repeated use. Though NIRS is a powerful imaging tool, it also has limitations, and methodological challenges that we addressed. What is of main importance, and which is what we have tried to point out herein, is that the development of NIRS techniques has made objective measures available that allow us to directly monitor relevant changes of localized regions in the brain induced by challenging motor and/or cognitive tasks.

References

[1] F. Jöbsis, Science 198 (4323) (1977) 1264–1267.
[2] H. Obrig, C. Hirth, J.G. Junge-Hülsing, C. Döge, T. Wolf, U. Dirnagl, A. Villringer, J. Appl. Physiol. 81 (3) (1996) 1174–1183.
[3] B. Chance, Z. Zhuang, C. UnAh, C. Alter, L. Lipton, Proc. Natl. Acad. Sci. USA 90 (8) (1993) 3770–3774.
[4] P.L. Madsen, N.H. Secher, Prog. Neurobiol. 58 (6) (1999) 541–560.
[5] B. Chance, Rev. Sci. Instr. 22 (1951) 634–638.
[6] A. Villringer, B. Chance, Trends Neurosci. 20 (10) (1997) 435–442.
[7] G. Strangman, D.A. Boas, J.P. Sutton, Biol. Psychol. 52 (2002) 679–693.
[8] P. van der Zee, M.E. Essenpreis, D.T. Delpy, Proc. SPIE 1888 (1993) 454–465.
[9] M. Cope, Med. Biol. Eng. Comput. 26 (3) (1988) 289–294.
[10] R.A. Shaw, J.R. Mansfield, V.V. Kupriyanov, H.H. Mantsch, J. Inorg. Biochem. 79 (1-4) (2000) 285–293.
[11] D.T. Delpy, M. Cope, P. van der Zee, S. Arridge, S. Wray, J. Wyatt, J. Phys. Med. Biol. 33 (12) (1988) 1433–1442.
[12] P. van der Zee, S.R. Arridge, M. Cope, D.T. Delpy, Adv. Exp. Med. Biol. 277 (1990) 79–84.
[13] S. Matcher, P. Kirkpatrick, K. Nahid, M. Cope, D.T. Delpy, Proc. SPIE 2389 (1995) 486–495.
[14] C.E. Cooper, C.E. Elwell, J.H. Meek, S.J. Matcher, J.S. Wyatt, M. Cope, D.T. Delpy, Pediatr. Res. 39 (1) (1996) 32–38.
[15] A. Duncan, J.H. Meek, M. Clemence, C.E. Elwell, L. Tyszezuk, M. Cope, D.T. Delpy, Phys. Med. Biol. 40 (1995) 295–304.
[16] M. Ferrari, L. Mottola, V. Quaresima, Can. J. Appl. Physiol. 29 (4) (2004) 463–487.
[17] H.R. Heekeren, M. Kohl, H. Obrig, R. Wenzel, W. von Pannwitz, S.J. Matcher, U. Dirnagl, C.E. Cooper, A. Villringer, J. Cereb. Blood Flow Metab. 19 (6) (1999) 592–603.
[18] A. Maki, Y. Yamashita, Y. Ito, E. Watanabe, Y. Mayanagi, H. Koizumi, Med. Phys. 22 (12) (1995) 1997–2005.
[19] E. Watanabe, Y. Yamashita, A. Maki, Y. Ito, H. Koizumi, Neurosci. Lett. 205 (1) (1996) 41–44.
[20] K.K. McCully, T. Hamaoka, Exerc. Sport Sci. Rev. 28 (3) (2000) 123–127.
[21] E.M. Sevick, B. Chance, J. Leigh, S. Nioka, M. Maris, Anal. Biochem. 195 (2) (1991) 330–351.
[22] T. Hamaoka, T. Katsumura, N. Murase, S. Nishio, T. Osada, T. Sako, H. Higuchi, Y. Kurosawa, T. Shimomitsu, M. Miwa, B. Chance, J. Biomed. Opt. 5 (1) (2000) 102–105.
[23] J. Choi, M. Wolf, V. Toronov, U. Wolf, C. Polzonetti, D. Hueber, L.P. Safonova, R. Gupta, A. Michalos, W. Mantulin, E. Gratton, J. Biomed. Opt. 9 (1) (2004) 221–229.
[24] H.H. Jasper, Electroencephal. Clin. Neurophysiol. 10 (1958) 371–375.
[25] E.K. Miller, J.D. Cohen, Annu. Rev. Neurosci. 24 (2001) 167–202.
[26] M. Suzuki, I. Miyai, T. Ono, I. Oda, I. Konishi, T. Kochiyama, K. Kubota, Neuroimage 23 (3) (2004) 1020–1026.
[27] P. Rasmussen, E.A. Dawson, L. Nybo, J.J. van Lieshout, N.H. Secher, A. Gjedde, J. Cereb. Blood Flow Metab. 27 (5) (2007) 1082–1093.
[28] N. Kuboyama, T. Nabetani, K. Shibuya, K. Machida, T. Ogaki, J. Physiol. Anthropol. Appl. Human Sci. 23 (4) (2004) 105–110.
[29] A. Vilringer, A.U. Dirnagl, Cerebrovasc. Brain Metab. Rev. 7 (3) (1995) 240–276.
[30] G. Gratton, M.R. Goodman-Wood, M. Fabiani, Hum. Brain Mapp. 13 (2001).
[31] A. Kleinschmidt, H. Obrig, M. Requardt, K.D. Merboldt, U. Dirnagl, A. Villringer, J. Frahm, J. Cereb. Blood Flow Metab. 16 (1996) 817–826.
[32] G. Strangman, J.P. Culver, J.H. Thompson, D.A. Boas, Neuroimage 17 (2002) 719–731.
[33] M. Hiraoka, M. Firbank, M.E. Essenpreis, M. Cope, S.R. Arridge, Phys. Med. Biol. 38 (1993) 1859–1877.
[34] L. Skov, O. Pryds, G. Greisen, Pediatr. Res. 30 (1991) 570–573.
[35] C.W. Yoxall, A.M. Weindling, N.H. Dawani, I. Peart, Pediatr. Res. 38 (1995) 319–323.
[36] T. Binzoni, T.S. Leung, D. Boggett, D. Delpy, Phys. Med. Biol. 48 (15) (2003) 2527–2549.
[37] T. Shiga, K. Yamamoto, K. Tanabe, Y. Nakase, B. Chance, J. Biomed. Opt. 2 (1997) 154–161.
[38] G. Jasdzewski, G. Strangman, J. Wagner, K.K. Kwong, R.A. Poldrack, D.A. Boas, Neuroimage 20 (2003) 479–488.
[39] G. Taga, K. Asakawa, A. Maki, Y. Konishi, H. Koizumi, Proc. Natl. Acad. Sci. USA 100 (2003) 10722–10727.
[40] I.T. Jolliffe, Principal Component Analysis, Springer Verlag, New York, 1986.
[41] L.O. Christensen, P. Johannsen, T. Sinkjaer, N. Petersen, H.S. Pyndt, J.B. Nielsen, Exp. Brain Res. 135 (1) (2000) 66–72.
[42] J.M. Orgogozo, B. Larsen, Science 206 (4420) (1979) 847–850.
[43] Y. Hoshi, Psychophysiology 40 (2003) 511–520.
[44] H. Fukuyama, Y. Ouchi, S. Matsuzaki, Y. Nagahama, H. Yamauchi, M. Ogawa, J. Kimura, H. Shibasaki, Neurosci. Lett. 228 (3) (1997) 183–186.
[45] I. Miyai, H.C. Tanabe, I. Sase, H. Eda, I. Oda, I. Konishi, Y. Tsunazawa, T. Suzuki, T. Yanagida, K. Kubota, Neuroimage 14 (5) (2001) 1186–1192.
[46] J.M. Thornton, A. Guz, K. Murphy, A.R. Griffith, D.L. Pedersen, A. Kardos, A. Leff, L. Adams, B. Casadei, D.J. Paterson, J. Physiol. 533 (Pt 3) (2001) 823–836.
[47] K. Ide, A. Horn, N.H. Secher, J. Appl. Physiol. 87 (5) (1999) 1604–1608.
[48] T. Rupp, S. Perrey, Eur. J. Appl. Physiol. 102 (2) (2008) 153–163.

[49] Y. Bhambhani, R. Malik, S. Mookerjee, Respir. Physiol. Neurobiol. 156 (2) (2007) 196–202.
[50] M.I. Pereira, P.S. Gomes, Y.N. Bhambhani, Sports Med. 37 (7) (2007) 615–624.
[51] A.W. Subudhi, M.C. Lorenz, C.S. Fulco, R.C. Roach, Am. J. Physiol. Heart. Circ. Physiol. 294 (1) (2008) H164–H171.
[52] K. Shibuya, M. Tachi, Respir. Physiol. Neurobiol. 153 (3) (2006) 261–266.
[53] S. Racinais, D. Bishop, R. Denis, G. Lattier, A. Mendez-Villaneuva, S. Perrey, Med. Sci. Sports Exerc. 39 (2) (2007) 268–274.
[54] Y. Hoshi, H. Onoe, Y. Watanabe, J. Andersson, M. Bergström, A. Lilja, B. Långström, M. Tamura, Neurosci. Lett. 172 (1-2) (1994) 129–133.
[55] M.K. Dalsgaard, J. Cereb. Blood Flow Metab. 26 (6) (2006) 731–750.
[56] E.Z. Ross, N. Middleton, R. Shave, K. George, A. Nowicky, Exp. Physiol. 92 (2007) 417–426.
[57] M.E. Raichle, A historical and physiological perspective, in: R. Cabeza, A. Kingstone (Eds.), Handbook of Functional Neuroimaging of Cognition, Massachusetts Institute of Technology Press, Cambridge, 2001, pp. 3–26.
[58] J.B. Pochon, R. Levy, J.B. Poline, S. Crozier, S. Lehéricy, B. Pillon, B. Deweer, D. Le Bihan, B. Dubois, Cereb. Cortex. 11 (3) (2001) 260–266.
[59] M.M. Lorist, D. Kernell, T.F. Meijman, I. Zijdewind, J. Physiol. 545 (2002) 313–319.
[60] A. Dietrich, Conscious Cogn. 12 (2) (2003) 231–256.
[61] A. Dietrich, P.B. Sparling, Brain Cogn. 55 (3) (2004) 516–524.
[62] N.M. Benwell, M.L. Byrnes, F.L. Mastaglia, G.W. Thickbroom, Neurosci. Lett. 167 (2005) 160–164.
[63] H. Van Duinen, R. Renken, N. Maurits, I. Zijdewind, Neuroimage 35 (2007) 1438–1449.