Review

Molecular concentration of deoxyHb in human prefrontal cortex predicts the emergence and suppression of consciousness

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Abstract

This is the first study to use fNIRS to explore anaesthetic depth and awakening during surgery with general anaesthesia. A 16 channel continuous wave (CW) functional near-infrared system (fNIRS) was used to monitor PFC activity. These outcomes were compared to BIS measures. The results indicate that deoxyHb concentration in the PFC varies during the suppression and emergence of consciousness. During suppression, deoxyHb levels increase, signalling the deactivation of the PFC, while during emergence, deoxyHb concentration drops, initiating PFC activation and the recovery of consciousness. Furthermore, BIS and deoxyHb concentrations in the PFC display a high negative correlation throughout the different anaesthetic phases. These findings suggest that deoxyHb could be a reliable marker for monitoring anaesthetic depth, and that the PFC intervenes in the suppression and emergence of consciousness.

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Introduction

Human consciousness is an ever-growing topic of interest in the field of neuroscience. In this article, we focus on the contribution of the human prefrontal cortex (PFC) to the emergence and suppression of consciousness. There are two main approaches to the psychophysiological study of human consciousness, one focusing on arousal and the thalamic-cortical system (Schiff, 2008; Tononi, 2004), and the other on the actual content of consciousness and how it relates to the cortico-cortical system (Dehaene et al., 2003; Gaillard et al., 2009; Sergent, and Dehaene, 2004). The former argues that different subcortical structures, including brainstem and thalamic nuclei, modulate consciousness state. This approach to the study of consciousness focuses primarily on the arousal needed by an individual to respond adequately to his/her environment. The second approach suggests that cortico-cortical networks define the content of consciousness. Fully preserved consciousness (or awareness) cannot exist without the participation of cortico-cortical networks, with the PFC as one of its regions of interest (León-Domínguez et al., 2013). These networks allow the conscious person to report on his/her mental state. From a neuropsychological standpoint, consciousness has a clearly defined behavioural component, both motor and intentional, which is associated with the integrity of these cortico-cortical networks.

There appears to be agreement that different cerebral processing systems, namely cognitive binding, permit long distance functional connectivity and extensive synchronised neural networks which generate conscious experience or awareness (Alkire et al., 2000; Baars, 1998, 2005; Bodizitz, 2008; Braun et al., 1997; Crick and Koch, 2003; Dehaene and Naccache, 2001; Dehaene et al., 2003; Gaillard et al., 2009; John, 2005; Kriegel, 2007; Leon-Carrion et al., 2012; Sergent, and Dehaene, 2004; Thagard and Aubei, 2008). Cognitive binding results from the large-scale synchronisation of spatially discrete neural subpopulations which appear to reflect the deeper relationships between consciousness, time and space. Both subcortical–cortical and cortico-cortical connections seem to be involved in the generation of synchrony, while binding most likely is essential for cognitive activities ranging from lower order processes to consciousness itself (Leon-Carrion et al., 2012; Mashour, 2004). Recent research suggests that the fronto-parietal network is responsible for modulating conscious behaviour by means of specific forebrain circuit mechanisms (Laureys and Schiff, 2012; Leon-Carrion et al., 2012). Other studies relate decreased activity in fronto-parietal networks and the PFC to a reduced consciousness level after the administration of anaesthetic drugs (John and Prichep, 2005). These works provide ample evidence of PFC intervention in consciousness. Although numerous investigations link hypoxia in the fronto-parietal network to anaesthesia-induced amnestic effects (Veselis et al., 2002) and nonconscious states (John and Prichep, 2005), we did not find evidence in the literature of clearly defined roles for the PFC in the emergence and suppression of consciousness. Indeed, some authors suggest that the level of synchronisation between anterior and posterior cortex defines a subject’s awareness of his/her surroundings (Leon-Carrion et al., 2012).

Other cerebral structures play an important role in consciousness – the thalamus intervenes by means of cognitive binding (Schiff, 2008) – yet it is the connection of these structures to the PFC that modulates an individual’s functional conscious state (Gaillard et al., 2009; León-Domínguez et al., 2013; Zikopoulos and Barbas, 2006, 2007). Conscious processes are directly related to high-order processes involving the PFC, including attention and working memory. These cognitive processes automatically choose cortico-cortical networks which are necessary for conscious manipulation of information and generating an adequate response to a given context (Baars and Franklin, 2003; Lamme, 2003; Posner and Petersen, 1990). Different studies point out that the PFC is involved in unconscious information processing, paradoxically in tasks necessary for consciousness, including cognitive control, response inhibition, task switching, conflict monitoring, and error detection (León-Carrion et al., 2008; van Gaal and Lamme, 2012). According to Baars (2005), “full consciousness may not exist without the participation of such prefrontal self systems.”

The goal of the present study is to clarify this issue by measuring PFC activity during the induction and removal of general anaesthesia. We used a 16 channel continuous wave (CW) functional near-infrared system (FNIRS) to monitor PFC activity during surgery with general anaesthesia. To monitor the rise and fall of consciousness, we compared FNIRS data to Bispectral Index measures (BIS; Aspect Medical Systems Inc., Natick, MA) (Sigl and Chamoun, 1994), the current gold standard for monitoring anaesthetic depth or hypnosis (Drummond, 2000; Johansen, 2006; Johansen and Sebel, 2000; Kissin, 2000). Unlike functional magnetic resonance imaging (fMRI), FNIRS facilitates the measuring of cortical activation in clinical environments (Izzetoglu et al., 2004). Following the seminal work of Jobis (1977), FNIRS has been successfully applied in research on changes in cerebral areas associated with motion, vision, audition, attention, memory, emotional and executive function, and involving various subject groups, including children, young and elderly adults, patient populations and healthy controls (Cannestra et al., 2003; Herrmann et al., 2008; Leff et al., 2011; Leon-Carrion et al., 2006a, 2006b, 2007; León-Carrion et al., 2008, 2010; León-Carrion et al., 2007; Nakahachi et al., 2010; Zaramella et al., 2001).

Other studies using classic neuroimaging techniques (PET and fMRI) in conjunction with FNIRS have shown that both techniques provide outcomes comparable to FNIRS (Cui et al., 2011; Hock et al., 1997; Huppert et al., 2006; Lee et al., 2008; Macintosh et al., 2003; Minati et al., 2011; Torovon et al., 2001, 2003; Villringer and Chance, 1997), even during resting state (Lu et al., 2010). FNIR technology measures two molecules, oxygenated haemoglobin (oxyHb) and deoxygenated haemoglobin (deoxyHb). We selected the deoxyHb molecule for cortical activation analysis due to its high correlation with the BOLD response in fMRI (Huppert et al., 2006; Macintosh et al., 2003; Torovon et al., 2001, 2003), and its higher discriminatory power over oxyHb in humans (Herrmann et al., 2008).

We chose a surgical setting for this study mostly because general anaesthesia’s hypnotics, sedatives and muscle relaxants leave the patient in an unconscious state optimal for this type of research. Moreover, non-random discharges due to muscle contraction or pain perception leave no remnants of cerebral activation. Thus, we avoid the risk of random residual activation or contamination during deep anaesthesia. The goal is to find the relevant PFC regions involved in the rise and fall of consciousness, and situate them within a neuropsychological context.

Materials and methods

This retrospective study assessed a total of 52 surgery patients. The final study sample included 20 patients, 17 male (85%) and 3 female (15%), who had undergone general anaesthesia during colorectal surgery. Mean age was 66.17 ± 17.2. Exclusion criteria included a lack of requisite data (7 patients), sevoflurane induced anaesthesia (3), no awakening from surgery and transfer to the ICU (2), under 18 years of age (3), a history of TBI (2), a history of stroke (6), and an excessive number of artefacts in their FNIRS recording (9), the latter primarily due to the noise generated by electrocautery. The study protocol was in accordance with the Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm), and approved by the Virgen del Rocio Hospital Ethics Committee. Written informed consent was obtained from all subjects.

Anaesthetic drug infusion

Anaesthetic induction was carried out via intravenous propofol infusion (Kanto and Gepts, 1989) and inhaled sevoflurane (Patel and Goa, 1996), respectively. Propofol, a GABA<sub>A</sub> receptor agonist, exerts its sedative and hypnotic effect through its agonist activity. This activity is also present at the glycine receptor and, in a milder form, at neuronal...
acetylcholine, AMPA and NMDA receptors (Rudolph and Antkowiak, 2003). Sevoflurane has agonist effects at GABA_A and glycine receptors (Franks, 2008). It is also an antagonist at NMDA, serotonin and AMPA receptors. In addition, sevoflurane has mild antagonist activity at acetylcholine receptors (Franks, 2008). Sevoflurane is otherwise very well tolerated and appears to offer the advantage of rapid and smooth induction and emergence from general anaesthesia (Young and Apfelbaum, 1995). If we compare the effects of sevoflurane and propofol on regional cerebral blood flow (rCBF) and the metabolic rate of oxygen (rCMRO2), propofol reduces rCBF and rCMRO2 comparably, while sevoflurane has less effect on rCBF and a similar effect on rCMRO2 (Kaisti et al., 2003).

Apart from hypnotic drugs, we also used remifentanil and cisatracurium, agents essential to surgery. Remifentanil is an analgesic drug (Troster et al., 2006), a short-acting, selective μ-opioid agonist with rapid onset and offset effects independent from the duration of infusion (Lee et al., 2010). Other opioid binding studies demonstrate that remifentanil has a strong affinity for the μ-opioid receptor and less so for delta and kappa receptors (James et al., 1991). Cisatracurium is a non-depolarizing neuromuscular blocking agent with an intermediate period of action (Kisor and Schmith, 1999). Non-depolarizing agents act by competing with acetylcholine for receptor sites on the motor endplate (Tuba et al., 2002).

### Anaesthetic effects on cerebral activity

The drugs used in the anaesthesia provide researchers with a stable reproduction of the temporal decrease or suppression of consciousness (Alkire et al., 2008). The use of anaesthesia and neuroimaging to study consciousness has traditionally been applied to help identify its neural correlates (Alkire, 2008). The neurophysiological effects of anaesthetics that produce amnesia and loss of awareness occur in six stages (John and Prichep, 2005):

**Stage 1.** The suppression of brainstem activity diminishes ascending reticular activating system (ARAS) activation of the thalamus and cortex. **Stage 2.** Depression of mesolimbic dorsolateral PFC interactions leads to a blockade of memory storage. **Stage 3.** Further depression of the ARAS releases its inhibition of the thalamic reticular nucleus, leading to hyperpolarizing GABA-mediated inhibitory action and resulting in the closure of thalamic gates (especially in the diffuse projection system). **Stage 4.** Reverberations of the cortico-thalamic system diminish. **Stage 5.** Cognition is blocked due to the disconnection of the fronto-parietal system. **Stage 6.** PFC activity is depressed to reduce awareness.

These stages illustrate how the loss of consciousness is gradual holistic process, with decreasing levels of PFC activity leading to the reduction of awareness.

### Experimental design

To study the rise and fall of consciousness under general anaesthesia, fNIRS and BIS data were collected simultaneously on the 23 subjects. fNIRS measures indicated the level of deoxyHb in the PFC, while BIS values showed the anaesthetic depth of each patient. Consciousness level was identified using fNIRS parameters and BIS values (0–100) (see Table 1). BIS measures are analysed, freed of artefacts, and compared to extensive data on anaesthetic depth. The BIS utilises a dynamic given that haemodynamic alterations differ between the two procedures. Laparoscopic techniques increase intra-abdominal pressure, and the supine position of the patient (Trendelenburg position) increases intracranial pressure. Our research design is based on the model proposed by John et al. (2001) for the study of anaesthetic-induced loss of consciousness (propofol for induction, sevoflurane for maintenance) We divided the experimental design assessing PFC activation into two stages, the pre-anaesthesia stage and the anaesthesia stage (see Fig. 1).

During the pre-anaesthesia stage, the anaesthesiologist reviews the patient’s current state and his/her medical history. Pre-operative data is collected, including age, gender, haemoglobin count, partial oxygen saturation, comorbidity and ASA (American Society of Anaesthesiologists Physical Classification System).

During the anaesthesia stage, haemodynamic data, oximetric parameters and fNIRS and BIS measures are recorded. Baseline is established during Phase 1. The subject, under the effects of midazolam (0.1–0.05 mg/kg), lies relaxed on a stretcher. A technician applies the fNIRS sensors in line with positions FP1–FP2 on the International 10–20 System, designed for recording data from the dIPFC (Izzetoglu et al., 2005). At this point, initial data from fNIRS, BIS and the subject’s haemodynamic state is recorded. Once baseline values are obtained, 3 minute pre-oxygenation begins (Flow = 10 L/min, FiO₂ = 0.5%).

**Phase 2 covers anaesthetic induction, beginning with remifentanil infusion (0.05–0.1 μg/kg/min) and followed by intravenous propofol infusion (2.0 mg/kg).** After loss of consciousness and face mask ventilation with oxygen, patients are given 0.2 mg/kg of cisatracurium. Three minutes later, the trachea is intubated, and the patient’s lungs are ventilated to maintain normocapnia (target end tidal CO₂: 35–40 mm Hg). Once BIS levels drop below 60, the anaesthesiologist verbally and physically stimulates the patient to ensure that he/she is under the effects of the anaesthesia. The second step involves tubing the patient for his/her mechanical ventilation; sevoflurane is administered to keep the patient under hypnosis.

**Phase 3 involves maintenance of anaesthesia depth.** Patients are given remifentanil (0.1–0.5 μg/kg/min), cisatracurium (1.5 μg/kg/min) and sevoflurane (1.5–2%) to an accepted level of hypnosis (BIS 40–60). During this phase, the patient is under deep anaesthesia and profoundly relaxed.

**Phase 4 covers light anaesthesia (anaesthesia removal).** During this phase, sevoflurane, cisatracurium and remifentanil are removed. The patient is said to “awaken” from the anaesthesia.

**Phase 5, the last phase of the study, is the moment of eye opening.** A recent study illustrated how patients emerging from deep anaesthesia first show signs of autonomic arousal, followed by a slow return of brainstem reflexes, eventually leading to reflexive or uncoordinated somatic movements that occur before subjects can willfully respond to simple commands (Langsjo et al., 2012). In keeping with this pattern of temporal and sequential connection of cerebral structures, in our study, the doctor calls out the patient’s name to confirm that he/she is conscious, and when eye opening occurs, the patient is asked to respond to simple commands, such as covering himself/herself with a blanket. During this phase, eye opening in response to the auditory stimulus and correct responses to these simple commands indicate that the patient is conscious and responding with adequate behaviour. The patient is then moved to the recovery room, or post-anaesthesia care unit (PACU).

Patients were kept under stable haemodynamic conditions throughout the surgical procedure. Analytic parameters as well as ventilation

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**Table 1**

<table>
<thead>
<tr>
<th>BIS values</th>
<th>Level of consciousness</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Awake</td>
</tr>
<tr>
<td>100–80</td>
<td>Responds to normal voice</td>
</tr>
<tr>
<td>80–60</td>
<td>Responds to loud commands or mild prodding/shaking</td>
</tr>
<tr>
<td>60–45</td>
<td>General anaesthesia, low probability of explicit recall, unresponsive to verbal stimuli</td>
</tr>
<tr>
<td>45–20</td>
<td>Deep hypnotic state</td>
</tr>
<tr>
<td>20–0</td>
<td>Burst suppression</td>
</tr>
<tr>
<td>0</td>
<td>Flat line EEG</td>
</tr>
</tbody>
</table>

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parameters were also maintained at normal values. Anaesthetic gases in our patient sample were similar in range, and percentages were adequate for anaesthetic depth, MAC (Minimum alveolar concentration), and % of sevoflurane (see Inline Supplementary Table S1).

fNIRS description

Functional near-infrared spectroscopy (fNIRS) is a neuroimaging modality that measures haemodynamic changes in the brain. Relative absorption and backscatter of near infrared light by oxyHb and deoxyHb reflect changes in neural activity via neurovascular coupling. Furthermore, by lending itself to small, noninvasive, inexpensive and portable lightweight devices, fNIR technology is increasingly applied in functional brain imaging studies.

The fNIRS probe is 17.5 cm long and 6.5 cm wide. It contains four light sources surrounded by ten detectors, for a total of 16 channels of data acquisition covering an area of 14 × 3.5 cm on the forehead. A source-detector distance of 2.5 cm provides a penetration depth of ≈1.25 cm. The probe positioning is such that the line of sources is set at the line of frontal polar electrodes (FP1–FP2 in the International 10–20 system), to image cortical areas that correspond to dlPFC (Izzetoglu et al., 2005). The dlPFC generally occupies the upper and side regions of the frontal lobes. It is comprised of BA 9 and 46. Area 9 occupies the dorsal region of lateral PFC and extends medially to the paracingulate of humans. Area 46 is generally located at the anterior end of the middle frontal sulcus. The frontal polar PFC, BA 10, is a region positioned above the orbital frontal cortex (oFC), inferior to Area 9, and anterior to Area 46, serving as a junction point between the oFC and dlPFC (Krawczyk, 2002). A complete data acquisition cycle lasts approximately 500 ms, making the temporal resolution approximately 2 Hz.

In the present study, we used a 16 channel continuous wave (CW) fNIR system (fNIR Devices LLC, Potomac, MD), which applies light to tissue at constant amplitude and can provide relative measurements of oxy- and deoxyHb. Although this system provides 16 channel recordings, only the upper 8 measurements could be used in this study (channels #1, #3, #5, #7, #9, #11, #13 and #15) (see Fig. 2). To collect BIS and fNIR recordings simultaneously for comparative assessment, BIS electrodes were attached to the frontal lobe in lower level fNIR channel locations, prohibiting the contact of fNIR light sources and detectors with the skin.

fNIRS data processing

Raw fNIRS data were filtered with a previously developed and adjusted finite impulse response low-pass filter (0.14–0.17 Hz) to eliminate possible heart pulsation, respiration artefacts, high frequency noise, etc. (Izzetoglu et al., 2005). Since the intrinsic nature of the haemodynamic signal under general anaesthesia may contain low frequency components, simple high-pass filtering was not applied to eliminate signal drifts or movement artefacts. Instead, we used a novel technique based on combined independent component analysis and principal component analysis (ICA/PCA), to remove environmental and equipment noise (caused by subjects, cables or sensor movement or due to BIS positioning and signal drifts) from the raw intensity measurements (Izzetoglu, 2008). The combined ICA/PCA method employs a data collection procedure where a dark current measure is obtained as a reference signal for the noise component separation from the source signal. Noise contamination in the data is identified by i) evaluating dark current measurement values above a certain intensity level (>420), and ii) correlating raw intensity measurements at 730 and 850 nm wavelengths, separately, with the dark current measurement.
Those above the present threshold (correlation coefficient \( R > 0.7 \)) are selected (Izzetoglu, 2008). The ICA and PCA methods are then applied separately to the selected noisy raw intensity measurements and the reference signals used as measurement signals. The best performing algorithm in noise suppression is based on the correlation between the noise removed outcome signal of both algorithms and the dark current signal. These correlations are performed separately and the smallest value is selected as the noise removed raw intensity measurement for use in oxyHb and deoxyHb calculations. Changes in deoxyHb relative to the 10-s baseline data collected prior to anaesthesia induction were calculated using a modified Beer–Lambert law (Cope and Delpy, 1988; Delpy et al., 1988).

Data epochs were extracted for the induction phase (1 min prior to induction and 4 min post-induction), deep anaesthesia phase (4 min prior to anaesthesia removal) and light anaesthesia phase (4 min prior to eye opening). Within each data epoch, 1 min deoxyHb averages were calculated with corresponding BIS values for further data presentation and statistical analysis.

**Data analysis**

Data were analysed using IBM 2.0 for Windows. Patients’ demographic and clinical data are expressed as mean ± standard deviation (see Table 2). DeoxyHb and BIS values during each anaesthetic phase are shown as medians and interquartile ranges (Q1 and Q3). Non-parametric analyses using the Wilcoxon rank-signed test were carried out to compare deoxyHb and BIS levels at 1 min prior and 4 min after anaesthetic induction (induction phase), and 1 min prior to anaesthetic removal and eye opening (emergence phase). Pearson correlation (\( r \)) was used to test linear associations between deoxyHb and BIS medians throughout the general anaesthesia. Alpha was set at 0.05 for all analyses.

Haemodynamic and oximetric data are shown in mean and standard deviation (see Inline Supplementary Table S1). Repeated-measures ANOVA was calculated to assess whether these variables changed during the phases of general anaesthesia. We used Spearman’s rank correlation (\( \rho \)) for linear association analyses between pre-operative deoxyHb and deoxyHb in anaesthetic phases, and between propofol dosage and deoxyHb in anaesthetic phases (see Inline Supplementary Table S2).

Changes in BIS and deoxyHb levels were represented using Z-scores. Z-scores were calculated by subtracting mean level at each phase from mean level at baseline and then dividing the results by their standard deviation at baseline.

**Results**

Table 2 displays pre-operative results during the pre-anaesthesia stage. Pre-operative haemoglobin showed 12.43 ± 2.26 g/dL. Partial oxygen saturation averaged 98.52 ± 1.48. In all cases, peripheral oxygenation saturations remained within safe anaesthetic range (95–100%), indicating that reduced oxygen supply was not a cause for changes in deoxyHb. BMI was situated at 27.11 ± 4.41 kg·m⁻², while 40% of the subjects showed ASA II (American Society of Anaesthesiologists), 55% showed ASA III and 5%, ASA I. Subjects’ medical histories showed that 45% had hypertension and 25% had diabetes type II. Only 20% had both AH and diabetes type II (see Table 2).

**fNIRS data analysis**

Fig. 3 displays example of single channel deoxyHb time course measurements during induction, deep and light anaesthesia. During post-induction, deoxyHb increases as compared to pre-induction values. DeoxyHb values remain stable during deep anaesthesia. During light anaesthesia, before eye opening, deoxyHb measures begin to drop.

Global medians for BIS and deoxyHb levels were calculated twice during each phase (induction and emergence). Medians, interquartile ranges and p-values are shown in Table 3. Median deoxyHb at baseline was 0.006 g/dL; at 4 min after propofol induction, it rose to 0.078 g/dL, a statistically significant increment (\( z = −3.97, p - value = 0.001 \)). During the emergence phase, deoxyHb fell from 0.08 g/dL at 1 min before anaesthetic removal, to 0.039 g/dL at 1 min prior to eye opening. This difference showed a tendency towards significance but did not reach significance (\( z = −1.76, p = 0.078 \)).

BIS values showed the highest significant differences between phases. At 1 min prior and 4 min after propofol induction, median values were 93 and 30.5, respectively (\( z = −10.97, p < 0.001 \)). Similar but inverse results were found when comparing 1 min prior to anaesthetic removal and 1 min prior to eye opening. BIS values increased from 47 to 84 (\( z = −10.93, p < 0.001 \)). DeoxyHb levels in the PFC were recorded throughout the surgery by the 8 fNIR channels. Median and interquartile values were calculated from the data obtained from each channel.

At baseline, deoxyHb levels ranged from 0.002 g/dL (channel 7) to 0.013 g/dL (channel 15). At 4 min after propofol infusion, deoxyHb levels rose in every channel except channel 1. However, only channels 7 and 11 showed significant differences between measures (channel 7: \( z = −3.024, p = 0.002 \); channel 11: \( z = −2.27, p = 0.023 \)).

In the emergence phase, deoxyHb levels showed a general decrease, as reflected in Table 3. However, 3 channels (#3, #5 and #15) show a slight increment, but only channel 11 showed a significant difference (\( z = −2.27, p = 0.023 \)).

The neuroanatomical location of channel 11 coincides with the right dorsomedial prefrontal cortex (dmPFC) (see Fig. 4). In the prior pilot study (Izzetoglu et al., 2011), most of the significant differences in deoxyHb between deep and light anaesthesia were on the right side (channel 12). Hence, the findings in this study are in agreement with our previous results.

**BIS and deoxyHb association**

Fig. 5 shows variations in deoxyHb and BIS levels compared to baseline values in each anaesthetic phase. All data are expressed in Z-scores. DeoxyHb levels increase during the first 4 min after propofol induction, remain stable during deep anaesthesia, and progressively decrease, approaching baseline values during the emergence phase.

The BIS pattern during these phases is the inverse of deoxyHb measures. BIS values drop sharply during induction, reaching a Z-score of 11.
and remain constant during deep anaesthesia. During the emergence phase, from 4 to 1 min prior to eye opening, BIS values progressively increased to near baseline levels. As shown in Fig. 5, absolute Z-scores for BIS are much higher than those of deoxyHb, indicating that BIS values undergo greater variation from baseline than deoxyHb.

A strong negative linear association between BIS and deoxyHb levels was confirmed using Pearson’s correlation (r = −0.8; p < 0.001) (see Fig. 6).

**Haemodynamic and oximetric parameters**

Systemic haemodynamic parameters alter during the different phases of anaesthesia (see Inline Supplementary Table S1). At baseline, mean heart rate was 78.45 ± 17.73, and arterial pressure averaged 101.95 ± 26.26. During the induction phase, heart rate remained stable (77.68 ± 19.36), although arterial pressure dropped to 74.74 ± 21.61. Oxygen supply averaged 47.08 ± 5.27, while oximetric data remained similar to deep anaesthesia levels. Arterial pressure during light anaesthesia rose slightly (80.39 ± 11.44), while oximetric data remained stable with a slight increase in oxygenation (56.09 ± 18.45) and oxygen flow (8.06 ± 5.32). Light anaesthesia etCO₂ settled at 31.34 ± 3.63, MAC dropped slightly (0.7 ± 0.22), and exhaled sevoflurane (1.07 ± 0.36%) was lower than during induction phase.

Inline Supplementary Table S1 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.07.023.

In the minutes prior to eye opening, heart rate rose to 83.54 ± 18.81, and arterial pressure reached pre-operative values (100.71 ± 23.78). However, oxygen supply (67.4 ± 28.09) and oxygen flow (9.37 ± 4.16) were higher than light and deep anaesthesia values. Lastly, etCO₂ reached 31.39 ± 3.55, while MAC and sevoflurane values decreased to 0.05 ± 0.08 and 0.07 ± 0.1, respectively (see Inline Supplementary Table S1). At the systemic level, differences between heart rate and blood pressure could have a high impact on neurovascularisation. The reduction of deoxyHb concentration in the PFC could be caused by the drop in heart rate and blood pressure after propofol perfusion. In any case, this data confirm that the return to baseline values is accompanied by re-established cerebral perfusion in the PFC, signalling the recovery of consciousness. Other research reports that the effects of propofol are not uniform and can be associated with structures linked to the functional circuits of arousal and awareness (Franks, 2008). This could be attributed to the fact that increases and decreases in blood pressure and heart rate have a general effect, whereas the mPFC and the dmPFC may require a different level of oxygen during the suppression and emergence of consciousness.

Any analysis of oximetric variables must keep in mind that these variables are more related to oxyHb than to deoxyHb, as the measure for assessing oxygen in the blood (Leon-Carrion and Leon-Dominguez, 2008). This is because deoxyHb measurements are more accurate than oxyHb measurements in the context of anaesthesia, especially when assessing changes in oxygen saturation. The use of deoxyHb provides a more reliable indicator of anaesthetic depth and emergence than oxyHb measurements.

**Table 3**

<table>
<thead>
<tr>
<th>Global values</th>
<th>Baseline</th>
<th>Induction phase</th>
<th>Emergency phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1° prior anaesthesia induction</td>
<td>4° after anaesthesia induction</td>
<td>1° prior anaesthesia removal</td>
<td>1° prior eye opening</td>
</tr>
<tr>
<td>DeoxyHb (g/dL)</td>
<td>0.006 (−0.01−0.02)</td>
<td>0.078 (−0.08−0.69)</td>
<td>0.001**</td>
</tr>
<tr>
<td>BIS</td>
<td>93 (88.5−97)</td>
<td>100.6 (26.5−43.5)</td>
<td>101.0**</td>
</tr>
<tr>
<td>DeoxyHb in each fNIR channel</td>
<td>0.006 (−0.01−0.02)</td>
<td>0.078 (−0.08−0.69)</td>
<td>0.001**</td>
</tr>
<tr>
<td>1</td>
<td>0.003 (−0.03−0.03)</td>
<td>−0.041 (−0.21−0.57)</td>
<td>0.911</td>
</tr>
<tr>
<td>3</td>
<td>0.008 (−0.03−0.05)</td>
<td>0.063 (−0.22−0.63)</td>
<td>0.478</td>
</tr>
<tr>
<td>5</td>
<td>0.004 (0.26)</td>
<td>0.362 (−0.01−0.65)</td>
<td>0.084</td>
</tr>
<tr>
<td>7</td>
<td>0.002 (−0.01−0.01)</td>
<td>0.134 (−0.68)</td>
<td>0.002**</td>
</tr>
<tr>
<td>9</td>
<td>0.005 (−0.04−0.01)</td>
<td>0.024 (−0.05−0.13)</td>
<td>0.204</td>
</tr>
<tr>
<td>11</td>
<td>0.011 (−0.02−0.03)</td>
<td>0.475 (−0.22−0.91)</td>
<td>0.023</td>
</tr>
<tr>
<td>13</td>
<td>0.01 (0.003)</td>
<td>0.042 (−0.03−0.75)</td>
<td>0.279</td>
</tr>
<tr>
<td>15</td>
<td>0.013 (−0.03−0.04)</td>
<td>0.062 (−0.05−0.75)</td>
<td>0.126</td>
</tr>
</tbody>
</table>

* p < 0.05.
** p < 0.01.

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DeoxyHb does not carry the oxygen molecule, so we could not directly interpret the impact of cerebral oximetry on deoxyHb, although future research efforts could investigate this further.

Other variables from the pre-surgery phase which could alter deoxyHb included pre-operatory haemoglobin and propofol dosage during anaesthetic induction. However, no significant correlations were found between these measures (see Inline Supplementary Table S2).

Behavioural results showed that while subjects did not respond to verbal and physical stimuli during Phase 2, they all obeyed simple commands at eye opening during Phase 5 (e.g., stick out your tongue, cover yourself with a blanket). Correct responses indicated that the patient was aware and in tune with his/her environment. Attention processes would be necessary for subjects to understand these simple commands. Behavioural data coincides with that of BIS, showing that patients were not conscious in Phase 2 (BIS = 30.5) and recovered consciousness in Phase 5.

Fig. 4. Channel #11 showed the highest activation during the emergence of consciousness. Red indicates right dorsomedial PFC.

Fig. 5. DeoxyHb and BIS levels compared to baseline values in each anaesthetic phase, expressed in Z-scores. The graph shows that deoxyHb levels increase during the first 4 min after propofol induction, remain stable during deep anaesthesia, and progressively decrease after sevoflurane removal, approaching baseline values during the emergence phase. The BIS pattern is the inverse of deoxyHb measures. Absolute Z-scores for BIS are much higher than those of deoxyHb, indicating that BIS values undergo greater variation from baseline than deoxyHb.

Fig. 6. Scatter plot of BIS and deoxyHb values in each anaesthetic phase. A strong negative linear association between BIS and deoxyHb levels was confirmed using Spearman’s rank correlation ($r = -0.8; p < 0.001$).

Inline Supplementary Table S2 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.07.023.
Phase 5 (BIS = 84.5). In our study, auditory consciousness is contingent on eye opening, as well as on positive responses to the anaesthesiologist's simple commands. However, we have no physiological measures to indicate when the subject recovers auditory consciousness, and if this precedes visual consciousness. This could be investigated in future research.

**Discussion**

In general, deoxyHb concentration in PFC showed significant increases during the induction phase. By contrast, a non-significant decrease was observed during the emergence of consciousness, although with a tendency towards significance (see Table 3). This result could be due to operating room procedures, whereby fNIRS is quickly disconnected when the patient recovers consciousness, so that he/she can be moved to the post-anaesthesia care unit. During emergence, cerebral functioning is progressively restored, as interactions between sensory and high order processing gradually recover (Liu et al., 2011). If the fNIRS equipment had been connected after eye opening, we would observe general activation in the PFC during the emergence of consciousness as high order cognitive functions are re-established.

From a neurophysiological standpoint, deoxyHb concentration in the PFC should be interpreted in light of the BIS data. During anaesthetic induction, BIS values drop, indicating that the patient’s consciousness is diminishing. During emergence, BIS values increase, as the patient progressively recovers consciousness (see Table 3). Thus, activity in the PFC is generally decreased during the induction phase, coinciding with an increase in deoxyHb and a drop in BIS. By contrast, during emergence, deoxyHb decreases while BIS values increase. Our behavioural and physiological measures show the patient recovering consciousness, but the statistical analysis indicates that the PFC is not significantly activated, although there is a tendency towards significance (see Table 3). Future research should continue fNIRS monitoring after eye opening, to confirm significant activation in the PFC during the emergence phase. It would also be useful to compare fNIRS measures after eye opening to neuropsychological measures to assess the recovery of high order cognitive functions.

During the induction phase, fNIRS data coincided temporally with that of BIS, indicating that when deoxyHb increases, PFC activity decreases, leading to the loss of consciousness. These results suggest that physiological activity in the PFC is reduced as consciousness is suppressed. During the emergence phase, deoxyHb levels are altered, showing an increase in some channels (channels #3, #5 and #15), and a decrease in others (channels #1, #7, #9, #11 and #13) (see Table 3). Both physiological and behavioural data from this phase confirm the partial recovery of consciousness, as evidenced by positive responses to the anaesthesiologist’s simple commands (stick out your tongue; cover yourself with the blanket). The apparent disorganisation of deoxyHb concentration in fNIRS channels could result from neural activation patterns responding to the neurophysiological changes that take place as high order cognitive functions are re-established (Leon-Carrion et al., 2006a, 2006b). Future research efforts should investigate whether or not this disorganisation continues after the recovery of these functions. Nevertheless, we observed a significant negative correlation between deoxyHb concentrations and BIS values (see Fig. 6). Our results also showed that PFC actively intervened in consciousness during anaesthetic induction and emergence. These results point out that a minimum of PFC activation is needed for consciousness to emerge.

Fig. 5 shows deoxyHb and BIS activity during the different anaesthetic phases. During minute 2 of post-induction, BIS values drop and deoxyHb levels peak, confirming the rapid anaesthetic effects of propofol on PFC activity (Fiset et al., 1999; John et al., 2001; Veselis et al., 2002, 2004). However, at minute 4 of the emergence phase, BIS values increase while deoxyHb levels remain high. At this point, during the emergence of consciousness, fNIR and BIS measures diverge, as BIS values identify the patient’s awakening from anaesthesia, and fNIR measures indicate that patient is still “asleep”. This apparent contradiction disappears 1 min later (3 min prior to eye opening), when deoxyHb values drop as BIS values increase. This could be a sign of divergence between physiological and haemodynamic brain activity during the emergence of consciousness. One possible explanation could be that electrophysiological activity “warns” neurovascular perfusion of the need to provide energy to regions where this activity is taking place, and hence the temporal delay in haemodynamic activation. From a clinical perspective, the reduction of deoxyHb concentration from min 4 to 3 before eye opening could be a physiological parameter that better predicts a patient’s eye opening and awakening than BIS values. Thus, deoxyHb values could prove more reliable than electrophysiological measures as a marker for predicting eye opening in these patients. All of these results would suggest that deoxyHb could be a reliable molecular indicator for anaesthetic induction and emergence. DeoxyHb could be useful in efforts to develop reliable depth of anaesthesia markers for surgical procedures with general anaesthesia.

We used behavioural and physiological variables to establish the subject’s state of consciousness during the aforementioned anaesthetic phases. Our data suggest that consciousness is present when PFC is activated, and decreases in line with PFC deactivation (see Table 3). During the induction phase, two PFC regions, left medial PFC (mPFC) and right dorsomedial PFC (dmPFC), showed the greatest deactivation. During the emergence phase, only right dmPFC showed significant activation (see Fig. 4).

We did not find studies in the literature which dissociated right and left dmPFC functions. The dmPFC, along with the ventral striatum and the nucleus accumbens, showed positive correlation with self-related processes, regardless of stimulus valence and intensity (Moran et al., 2006; Northoff et al., 2009). Keenan et al. (2000) suggest that right PFC activation is associated with self-processes. Right dlPFC is associated with mnemonic processes including the recovery of episodic memory (Gilboa, 2004; Tulving et al., 1994). Episodic memory is related to auto-ethnographic capacities, or the capacity to remember autobiographical moments from the past and restore them to the present so that they may intervene in cognition (Ferbinteanu et al., 2006). The suppression of consciousness may thus be related to the capacity to deactivate neural circuits that intervene in memory and self. Moreover, right mPFC, as part of the right dmPFC, is associated with sustained attention, a basic function that determines the efficacy of ‘higher’ aspects of attention (selective attention, divided attention), and cognitive capacity in general (Sarter et al., 2001). Sustained attention prepares an individual to respond in the absence of salient or novel external stimuli, which engage attention automatically. Hence, both regions are involved in mnemonic and attention processes (Burianova and Grady, 2007; Fletcher et al., 1997; Hsu and Price, 2007; Huang et al., 2006; Lee et al., 2000), which are widely related to conscious processes (Knudsen, 2007).

The PFC was chosen as region of interest given the extensive evidence of its participation in modulating awareness (John, 2005). The PFC should not be regarded as the main structure in the emergence of consciousness, but its activation, particularly that of the right dmPFC, should be considered an essential element of fully preserved consciousness. Del Cul et al. (2009) suggest that the PFC could be one of the key nodes which regulate access to consciousness. Our data suggest that the right dmPFC may be the key neural node with accesses to consciousness.

There are two limitations which should be mentioned in relation to this study. One is fNIRS’ traditionally low spatial resolution of brain activity. We resolved this by utilising a 16 channel fNIR system and the individual analysis of each channel’s measurements. Another difficulty was that studies which have used fNIRS to measure the cortical activity of evoked stimuli have found that the haemodynamic response produces a post-stimulus interval of 20–30 s. However, the nature of this study is very different than a task related study conducted in a laboratory; the data was analysed over a 4 min window but for 1 min intervals. Patients were not given external stimuli to generate an evoked
response. Furthermore, during the anaesthesia maintenance stage, we expected a stable haemodynamic response as opposed to an evoked response. Therefore, 1 min intervals are appropriate for the purpose of this study. However, in the future, 30 s and 20 s interval averages will also be analysed.

A second point to consider is that variation in fNIR measures may not only be due to consciousness related changes but also to systemic effects, such as changes in heart rate, CO₂ or oxygen saturation levels, as a response to anaesthesia. In order to eliminate such possible effects, a multidistance fNIR sensor can be used in the future. Larger source detector measurements, unlike smaller devices which only pierce non-cortical layers (skin, scalp, skull at most to CSF), can penetrate deeper and observe changes at cortical levels which may include consciousness-related variations together with systemic changes. Smaller source detector measurements can then be used to suppress these systemic effects and reach consciousness-related effects only.

Conclusion

This is the first study to use fNIRS to assess anaesthetic depth and awakening. In general, global levels of deoxyHb in the PFC vary throughout the different anaesthetic phases. While deoxyHb increases significantly during the induction phase, its levels show a tendency towards signification in the emergence phase. In a more in-depth analysis of PFC regions, only channels in the left mPFC and right dmPFC during induction, and in the right dmPFC during emergence showed significant differences. Furthermore, Bis and deoxyHb concentrations in the PFC showed a high negative correlation during the different phases of general anaesthesia. These results suggest that deoxyHb concentration in the PFC could be a reliable marker for monitoring anaesthesia depth, and that the PFC intervenes in the suppression and emergence of consciousness.

These results from fNIRS application should be considered provisional until further research can be done with larger samples sizes. These findings could lead to two lines of research. The first would be to use the deoxyHb molecule to measure anaesthetic depth during surgery with general anaesthesia. This type of monitoring could help improve existing techniques for anaesthetic depth control. The second is related to the study of disorders of consciousness, and the roles of the mPFC and dmPFC in the minimal conscious state, vegetative state, and coma. Further research is needed to determine which combination of fNIRS and fMRI across multiple cognitive tasks. NeuroImage 54, 2808–2821.


