Brain Auditory Activation Measured by Near-Infrared Spectroscopy (NIRS) in Neonates

PATRIZIA ZARAMELLA, FEDERICA FREATO, ANGELA AMIGONI, SABRINA SALVADORI,
PAOLA MARANGONI, AGNESE SUPPEI, BARBARA SCHIAVO, LINO CHIANDETTI

Department of Paediatrics, University of Padua, Via Giustiniani, 3, IT-35128 Padova, Italy

ABSTRACT

This study presents a new measure of the hemodynamic changes to an auditory stimulus in newborns. Nineteen newborns born at 28-41 wk and aged 1 to 49 d were studied in waking and/or sleeping state, for a median time of 4 min 40 s before, 2 min 40 s during, and 3 min 5 s after an acoustic stimulus (tonal sweep of frequency increasing from 2 to 4 kHz, intensity 90 dB SPL) originating 5 cm from the external auditory meatus. The emitter and detector optodes were placed over the left or right temporal region, corresponding to T3 or T4 EEG electrodes. The concentration changes in cerebral chromophores $\Delta[\text{HbO}_2]$, $\Delta[\text{Hb}]$ and Δ oxidized-reduced cytochrome aa_3 were recorded every 5 s. Changes in cerebral blood volume were calculated from the changes in total $\text{Hb} \times 0.89/\text{large vessel Hb concentration}$. Increased oxyhemoglobin, $\Delta[\text{HbO}_2]$, total Hb , $\Delta[\text{Hb}_{\text{sum}}]$, and cerebral blood volume, ΔCBV , were found in 13/19 neonates, with the exception of a neonate who only had increased in $\Delta[\text{Hb}]$, $\Delta[\text{Hb}_{\text{sum}}]$ and ΔCBV . During the stimulation phase there was a significant increase in ΔCBV (t test, $p = 0.00006$) in the responsive newborns from a mean value of $0.006 (\pm 0.02)$ mL/100 g in the pretest phase to $0.09 (\pm 0.06)$ mL/100 g during the auditory stimulus. After the test ΔCBV decreased to $0.04 (\pm 0.07)$ mL/100 g (t test, $p = 0.01$), so did $\Delta[\text{Hb}_{\text{sum}}]$ ($p = 0.02$). Hemodynamic responses of the subjects who showed increases

in $\Delta[\text{Hb}_{\text{sum}}]$ and $\Delta[\text{HbO}_2]$ were analyzed to study the $\Delta[\text{Hb}]$. The responder subjects could be classified into two groups according to $\Delta[\text{Hb}]$ changes: 8/13 (61.5%) showed an increase of $\Delta[\text{Hb}]$ (pattern A), while 5/13 (38.4%) showed a decrease (pattern B) (t test, $p = 0.03$). These two patterns did not show differences related to $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}_{\text{sum}}]$. The ΔCBV changes in nonresponders presented a decrease during the test phase (t test, $p = 0.04$). CBV did not return to pretest values, suggesting a fronto-temporal brain pathway for storing unusual sounds. The increase in CBV followed the local increase in oxyhemoglobin and total Hb concentrations due to a greater use of oxygen in the homolateral temporal cortex of the newborns. (*Pediatr Res* 49: 213-219, 2001)

Abbreviations:

$\Delta[\text{HbO}_2]$, oxyhemoglobin change
 $\Delta[\text{Hb}]$, deoxyhemoglobin change
 $\Delta[\text{CtOx}]$, cytochrome oxidase change
 $\Delta[\text{Hb}_{\text{sum}}]$, total Hb change
 ΔCBV , cerebral blood volume change
 BAEP , brainstem auditory evoked potentials
 NIRS , near-infrared transmission spectroscopy

Near-infrared transmission spectroscopy (NIRS) through tissue is made possible by the transparency of biologic tissue to light in the near-infrared region (750-1000 nm). The noninvasive nature of the technique and the portability of the device, combined with the relative transparency of the neonatal head, explain the early applications of the machine in neonatal intensive care units, to the observation of hemodynamic brain responses. Our study presents a method for applying NIRS to the testing of central auditory response in newborns by using an acoustic stimulus, a tonal sweep with a frequency increasing from 2 to 4 KHz. The incident near-infrared light from the transmitting optode is scattered through the tissues, and the reflected light is read by a receiving optode. The amount of the

light that a compound absorbs is dependent upon the wavelength of the incident light upon it, and is described by the spectrum of a single compound. Brain absorption of the light is due to the main cerebral chromophores, oxyhemoglobin (HbO_2), deoxyhemoglobin (Hb), and oxidized cytochrome oxidase (CtOx) and is determined by the oxygenation status of the compound. Through NIRS we measured the loss of the intensity due to absorption of the incident photons by these solutes; this loss or attenuation is usually measured in units of OD (OD) and can be calculated using the Beer-Lambert law. For an absorbing compound dissolved in a nonabsorbing medium, the attenuation (A) is proportional to the concentration of the compound in the solution (c), the specific extinction coefficient of the compound (α) and the optical path length (d). The loss of light intensity (attenuation), $A = \lg [I_0/I] = \alpha c d$. (A = attenuation measured in OD; I_0 = the light intensity incident on the medium; I = the light intensity transmitted through the

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Patrizia Zaramella, M.D., Department of Paediatrics, Neonatal Intensive Care Unit, University of Padua, Via Giustiniani, 3, IT-35128 Padova, Italy.

medium; α = specific extinction coefficient of the chromophore measured in $\mu\text{mol}^{-1} \cdot \text{L}^{-1} \cdot \text{cm}^{-1}$; c = the concentration of the chromophore in the solution measured in $\mu\text{mol} \cdot \text{L}^{-1}$; d = distance between the points where the light enters and leaves the solution measured in centimeters). It has been estimated that with sensitive instrumentation a NIR light penetration up to 8 cm is possible in a biologic tissue from the sending to the receiving points (1). The scattering effect of the light increases the attenuation at a single sample point above that to be expected from absorption alone. When the light is absorbed the energy of photons is lost as thermal energy throughout the absorber. Because the photon collisions are elastic, no energy is lost when NIR light is scattered in tissue, and the photons change direction. In the brain tissue there are known to be some microscopic and macroscopic boundaries (*i.e.* cell membranes, bone, and large blood vessels) which can increase scattering. The scattering effect of tissue explains why the optical pathlength is much greater than the geometrical distance (d) between the sites of light entry and exit. This true optical distance has been named as differential pathlength (DP) and the scaling factor as the differential pathlength factor (DPF). The absolute concentration of a chromophore is unknown, and the measurements are expressed as absolute concentration changes from an arbitrary zero made at the beginning of the measurements. Using this technique the changes in tissue oxygenation can be noninvasively monitored. This relative change can be used to calculate the absolute changes in cerebral blood flow and cerebral blood volume through the increase of a known amount of FiO_2 . Experimental data on the neonatal head demonstrate that contamination of the light signals due to transmission through skin and bone is minimal when the inter-optode spacing is more than 3 cm (2). The fibers for emitting NIR light are optical fibers terminating as small cylindrical optodes containing prisms that direct the light normally onto the surface of the tissue because the highly scattering brain medium is scanned between the two optodes. The inter-optode distance consequently means the inter-optode spacing, and this distance is the straight line, rather than the curve, through the two points. This is due to the light's diffusion in the brain, which acts as an isotropic source (3), even though the angle of the emission-detection lines is less than 180° .

NIRS has been used to demonstrate evoked changes in regional hemodynamics in adults and in infants after sensorineural stimulation (Table 1) (4–12). The increase in cerebral blood volume (CBV) follows the increase in HbO_2 (oxyhemoglobin) and in Hb (deoxyhemoglobin) seen in the occipital region upon visual stimulation. Changes in both HbO_2 and in Hb are measured from an arbitrary zero, and their sum is equal to total Hb, which is related to changes in CBV (13). Ohnishi *et al.* (9) first measured the changes evoked in the hemodynamics of the auditory cortex with NIRS and magnetoencephalography in an adult subject. They found significant increases of the total Hb and deoxyHb in the brain region at a depth of 20–30 mm corresponding to the auditory cortex of the MEG topogram. Increased oxyhemoglobin and total Hb induced by auditory (music) stimulation were found in the bilateral frontal lobes of the newborns studied recently by Sakatani *et al.* (12). They described two patterns with and without an increase of Hb (deoxyhemoglobin). To study the auditory function of the newborns, we performed an NIRS application in monitoring the changes of oxygenation as well of cerebral blood volume in the temporal cortex of 19 preterm and term neonates stimulated with a tonal sweep sound with a frequency increasing from 2 to 4 KHz.

SUBJECTS AND METHODS

Nineteen newborns aged 1 to 49 d (median 15 d) were studied. They were born between 1998 and 2000 and were admitted to the Neonatal Unit at the Pediatric Department of Padua University at birth. The mean gestational age was 32 wk (range 28 to 41 wk) and the averaged birth weight was 1936.7 g (range 990 to 4500 g). The diagnosis on admission was jaline membrane disease (5 neonates), congenital heart disease (2 neonates), twin pregnancy and/or apneic spells, with or without metabolic problems (16 neonates), asphyxia (1 neonate). Six newborns were incubated and ventilated at birth for a time ranging between 1–7 d, and the median hospital stay at the NICU was 21 d (range 10 to 57 d). Ten patients were treated with oxygen therapy for a mean time of 5 d (range 1 to 8 d). All infants, except one neonate with subependymal hemorrhage, appeared normal on cranial ultrasound or had transient periventricular echodense areas at the time of study (HDI 3000 CV,

Table 1. Sensorineural stimulation and brain evoked activation NIRS studies

Study	Stimulus	Localization of probes	Stimulus/ rest duration	Mean $\Delta[\text{Hb O}_2]$ (SD) ($\mu\text{mol/L}$)	Mean $\Delta[\text{Hb}_{\text{sum}}]$ (SD) ($\mu\text{mol/L}$)	Mean $\Delta[\text{Hb}_{\text{sum}}]$ (SD) ($\mu\text{mol/L}$)	Mean ΔCBV (SD) ml/100 g
Villringer A, <i>et al.</i> , 19934	Flashlight	Right occipital	1 min	+1.16 (0.26)	−0.67 (0.51)	+0.49 (0.74)	
Villringer A, <i>et al.</i> , 19934	Picture observation	Right occipital	1 min	+2.78 (2.54)	−2.57 (0.61)	+0.18 (2.9)	
Hoshi Y, <i>et al.</i> , 19935	Flashlight	Occipital	2–3 min	+	+	+	
Hoshi Y, <i>et al.</i> , 19935	Auditory (music)	Temporal	2–3 min	+	+	+	
Kato T, <i>et al.</i> , 19936	Flashlight	Besidesinion	10/10 min	3.4 (2.46)	+/0		
Meek JH, <i>et al.</i> , 1995, 1998	Moving colored disks	Below and beside inion	30/30 s	+0.54 (0.14)	+0.06 (0.08)	+0.61 (0.21)	+0.04 (0.01)
Ohnishi M, <i>et al.</i> , 19979	Tone burst sine wave	Left temporal lobe	50 ms		+	+	
Heekeren HR, <i>et al.</i> , 199910	Visual stimulation	Occipital cortex	10/30 s	+0.12 (0.03)	−0.10 (0.01)		
Bartocci M, <i>et al.</i> , 199911	Olfactory stimulation	Orbital-frontal bilateral	10 s	−	=		
Sakatani K, <i>et al.</i> , 199912	Auditory (piano music)	Frontal bilateral	10 min	+	+/−	+	
Present study	Auditory (sweep)	Temporal unilateral	2 min 40 s	+1.08 (1.21)	+0.53 (1.31)	+1.62 (1.08)	+0.09 (0.06)

+, increased; −, decreased; +/−, increased or decreased; =, not increased and not decreased.

ATL, USA). All the neonates were in good condition, and normal care was provided at the time of the examination. Clinical details are given in Table 2. The emission and detection probes of the Near-Infrared Oxygenation Monitor (NIRO 300 Hamamatsu Photonics KK, Japan) were placed on the neonates' temporal area (inter-optode distance 3.5 cm) to study the cerebral cortex and adjacent subcortical white matter in one of the temporal auditory brain sites. A personal computer converted absorption changes at each wavelength, recorded every 5 s, into relative concentration changes in cerebral chromophores: $\Delta[\text{HbO}_2]$, $\Delta[\text{Hb}]$, Δ oxidized-reduced cytochrome aa₃. The changes were expressed in micromol/L, and the optical pathlength was calculated by multiplying the inter-optode distance by 3.9 DPF (14). The sum of changes in oxyhemoglobin and deoxyhemoglobin enabled the calculation of the changes in total Hb, $\Delta[\text{Hb}_{\text{sum}}]$; because the hematocrit was constant, changes in CBV (mL/100 g of brain tissue) were calculated from the changes in total Hb $\times$ 0.89/Hb concentration in g/dL, corresponding to a large-vessel Hb concentration (13, 15). Using double-faced adhesive tape (bordering the external ear, *i.e.* corresponding to a T3 or T4 placement of the 10/20 EEG system), the probe holders were placed over the left or right temporal region corresponding to the primary auditory cortex. To prevent ambient light from reaching the optodes, a dark felt covered the neonate's head. The neonates were studied while they were in sleeping and/or in waking states, in an interval covering a median time of 4 min 40 s before, 2 min 40 s during and 3 min 5 s after the hearing stimulus. Audio-metric sound stimulation was a tonal sweep with a frequency

increasing from 2 to 4 kHz, intensity 90 dB SPL (Electrical Acoustics Constructions, EAC, Milan Mercury) applied 5 cm away from the external auditory meatus. The reasons for choosing this particular auditory stimulus were to prompt more attention to a train of briefly repeated high-energy sounds rather than to a continuous sound of constant energy (16, 17). Movement artifacts were seen during the study period, but we ruled out any influence of the consequent sharp spikes on the chromophores by excluding the traces containing the spikes in relation to a cytochrome aa₃ threshold level between 0.2 and -0.2 (18). On excluding the changes in chromophores related to the artifact movements, the evaluation of the test-phase was calculated in a time ranging from 80 (16 changes) to 430 (86 changes) seconds. Brainstem auditory evoked potentials (BAEPs, Multibasis System 2791, Italy) were performed in the newborns at an age spanning from 12 d to 9 mo. Student's *t* test was performed to compare the averaged NIRS parameters in relation to the studied phases. Ethical approval for the study from the local ethics committee and informed consent from parents were obtained.

RESULTS

The concentration changes in $\Delta[\text{HbO}_2]$, $\Delta[\text{Hb}]$, $\Delta[\text{Hb}_{\text{sum}}]$, and $\Delta[\text{CtOx}]$ are in micromol/L; CBV is given in mL/100 g of brain tissue. The baseline or pretest values represent the chromophore changes and CBV at the normal/basal condition of the neonates. We considered the averaged changes and the CBV changes in the basal, stimulus, and poststimulus phases. "Zero

Table 2. Clinical details of neonates

Neonate	Delivery	GA*	Apgar score	BW†	Age at NIRS (days)	Cranial ultrasound	Admission diagnosis
1	CS‡	34	9,9, 10	3020	6	Periventricular echodensities	HMD§
2	CS	32	9,10, 10	1990	1	Normal	Twin, hypoglycemia
3	CS	32	7,9, 10	2890	2	Normal	Twin, hypoglycemia
4	CS	28	9,10, 10	990	30	Normal	Hyperbilirubinemia, transient myocardial ischemia
5	CS	29	5,7, 10	1450	20	Normal	Asphyxia, hyperbilirubinemia
6	CS	33	9,10, 10	2240	2	Plexus cyst	Hypoglycemia, metabolic acidosis
7	vaginal	41	8,9, 10	4500	2	Normal	Great vessels transposition
8	CS	31	7,9, 10	1630	30	Normal	HMD, patent ductus arteriosus, hyperbilirubinemia
9	vaginal	41	8,9, 10	4400	17	Normal	Great vessels transposition
10	CS	31	7,9, 10	1155	17	Normal	Twin, hypoglycemia
11	CS	32	10,10, 10	1695	1	Normal	Hypoglycemia
12	CS	31	8,9, 10	1390	17	Plexus cyst	Twin, apneic spells
13	CS	31	7,9, 10	1490	17	Periventricular echodensities	Twin, hypoglycemia, apneic spells
14	CS	31	7,9, 10	1700	17	Periventricular echodensities	HMD, twin
15	CS	33	9,10, 10	1800	9	Periventricular echodensities	Hypoglycemia, apneic spells
16	CS	31	8,9, 10	1390	22	Normal	Hypoglycemia, transient myocardial ischemia
17	vaginal	32	7,9, 10	2100	12	SEH	Apneic spells, hypoglycemia, hypocalcemia
18	CS	33	7,7, 7	2290	14	Normal	HMD, patent ductus arteriosus, hyperbilirubinemia
19	CS	33	7,8, 10	1140	49	Periventricular echodensities	HMD, patent ductus arteriosus, apneic spells, hyperbilirubinemia

* GA, gestational age (weeks).

† BW, birth weight (grams).

‡ CS, cesarean section.

§ HMD, hyaline membrane disease.

set" reported to baseline (corresponding to 0 change), the chromophore changes before each phase of the study. An increased $\Delta[\text{Hb}_{\text{sum}}]$ and ΔCBV with respect to the baseline was found in reaction to the auditory stimulus in 13 of the neonates. All of these neonates revealed an increased $\Delta[\text{HbO}_2]$ except for neonate number 5. On the basis of these patterns, the neonates were arbitrarily considered as responders. Mean $\Delta[\text{HbO}_2]$ values increased from $0.06 (\pm 0.41) \mu\text{mol/L}$ before to $1.08 (\pm 1.21)$ during the test. $\Delta[\text{Hb}]$ changes increased from $0.05 (\pm 0.35)$ to $0.53 (\pm 1.31) \mu\text{mol/L}$ in the test as did $\Delta[\text{Hb}_{\text{sum}}]$ from $0.11 (\pm 0.34)$ to $1.62 (\pm 1.08)$. The comparison between pretest *versus* test values of chromophores (*t* test) in responsive newborns had shown a significant increase of $\Delta[\text{HbO}_2]$, ($p = 0.008$) and $\Delta[\text{Hb}_{\text{sum}}]$, ($p = 0.0001$) (Fig. 1). The remaining 6 neonates revealed a decreased $\Delta[\text{HbO}_2]$ (average values from 0.07 ± 1.1 to $-1.21 \pm 0.35 \mu\text{M}$, $p = 0.007$, *t* test), $\Delta[\text{Hb}]$ (average values from -0.27 ± 0.27 to $-0.99 \pm 0.96 \mu\text{M}$, NS, *t* test) and $\Delta[\text{Hb}_{\text{sum}}]$ (average values from -0.2 ± 1.16 to $-2.2 \pm 0.32 \mu\text{M}$, $p = 0.06$, *t* test), because they were considered as nonresponders (Fig. 2). $\Delta[\text{Hb}_{\text{sum}}]$ enabled the calculation of cerebral blood volume changes in the phases studied. During the stimulation phase there was a significant increase of ΔCBV (*t* test, $p = 0.00006$) in the responsive newborns from a mean value of $0.006 (\pm 0.02) \text{ mL}/100 \text{ g}$ in the pretest to $0.09 (\pm 0.06) \text{ mL}/100 \text{ g}$ during the auditory stimulus (Fig. 3). After the test (Fig. 4) ΔCBV decreased to $0.04 (\pm 0.07) \text{ mL}/100 \text{ g}$ ($p = 0.01$). ΔCBV changes in nonresponders presented a decrease during the test phase (*t* test, $p = 0.04$) (Fig. 5) and no differences were found when the chromophore mean poststimulus values were compared with the stimulus phase. The same comparison in the responders demonstrated a decreasing $\Delta[\text{Hb}_{\text{sum}}]$ poststimulation ($p = 0.02$). Hemodynamic response of the subjects who showed increases in $\Delta[\text{Hb}_{\text{sum}}]$ and $\Delta[\text{HbO}_2]$ were analyzed to study the $\Delta[\text{Hb}]$ changes. The responsive subjects could be classified into two groups according to $\Delta[\text{Hb}]$ changes: 8/13 (61.5%) showed an increase of $\Delta[\text{Hb}]$ (pattern A), while 5/13 (38.4%) showed a decrease (pattern B) (*t* test, $p = 0.03$). These two patterns did not show differences related to $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}_{\text{sum}}]$ (Fig. 6).

Fig. 1. Comparison between pretest *vs* test values of chromophores (*t* test) in responsive newborns.

Fig. 2. Comparison between pretest *vs* test mean values of chromophores (*t* test) in unresponsive newborns.

Fig. 3. CBV changes in responsive newborns in relation to the stimulation phase (*t* test, $p = 0.00006$).

Fig. 4. CBV changes in responsive newborns (*t* test) in relation to the stimulation phase.

Brainstem evoked responses demonstrated that 3/13 of the responders showed abnormal patterns, compared with 1/6 of the nonresponders. The data on a single neonate are presented in Fig. 7: test phase is marked. During stimulation there is an increase in $[\text{HbO}_2]$ and $[\text{Hb}_{\text{sum}}]$ (red and green lines), which decrease when the stimulus is removed. $[\text{Hb}]$ (blue line) decreases during test phase and cytochrome oxidase (yellow line) does not change significantly.

DISCUSSION

Sensorineural NIRS studies have been performed to measure regional changes in the occipital cortex following visual stim-

Fig. 5. CBV changes in unresponsive newborns (t test) in relation to the stimulation phase.

Fig. 6. t Test between the mean values of the subjects with (pattern A, $n = 8$) and without (pattern B, $n = 5$) increase of Hb during the test.

Fig. 7. Tracing of a responsive neonate. Red line indicates the $\Delta[\text{HbO}_2]$ changes; blue line the $\Delta[\text{Hb}]$ as the green the $\Delta[\text{Hb}_{\text{sum}}]$. Yellow line corresponds to cytochrome oxidase changes. Arrows mark the test phase: an increasing $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}_{\text{sum}}]$ are shown, during the test phase; however, they decrease poststimulation. $\Delta[\text{Hb}]$ decreases during test phase and cytochrome oxidase does not change significantly.

ulation in adults and infants (7, 8). Only two adult case studies (5, 9) have been published on brain auditory cortex hemodynamics during the hearing function. On the basis of a magnetoencephalographic (MEG) and NIRS study, Ohnishi *et al.* (9) found the auditory brain area to be about 25 mm under the scalp. In a dynamic multichannel near-infrared adult study, Hoshi and Tamura (5) reported evoked auditory increases in $\Delta[\text{HbO}_2]$, $\Delta[\text{Hb}]$, $\Delta[\text{Hb}_{\text{sum}}]$, in a 28-yr-old man listening to classical music through earphones from a tape-recorder. Saka-

tani K *et al.* (12) had first measured NIRS changes, induced by auditory stimulation, in newborns. They found that frontal lobes show a neuronal response similar to those that have been described in the neonatal visual cortex. Particularly, they found an increase of $\Delta[\text{Hb}]$, associated with increase of the $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}_{\text{sum}}]$, supporting the hypothesis that O_2 -consumption (blood flow $\times$ arterial-venous oxygen contents) exceeds O_2 -delivery (blood flow $\times$ arterial oxygen content), during neuronal activity in neonates. Adult brain generally presents a reduced concentration of $\Delta[\text{Hb}]$ at the activating cortical area because O_2 delivery exceeds O_2 consumption (19–23). We studied the auditory response in the temporal area of 19 newborns. The scattering of infrared light in tissue made it impossible to define a precise anatomical border, however. A decline in the NIRS signal has been reported when the optode is misplaced 2 cm away from the motor cortex (7). This means that an NIRS signal can be recorded with an external band region 1–2 cm wide. The acoustic pathways to the cortex are complex. There is an exact correspondence between the cochlea and the acoustic area of the cortex (24). Corti's organ has a bilateral representation in the brain and the central pathways enable a distinction between the tonal frequency, intensity and origin of sounds. Auditory brainstem studies show a binaural interaction (25) that our study did not evaluate. In human adults magnetoencephalographic recordings demonstrate processing in the superior temporal plane of the auditory cortex when a novel sound is administered over the acoustic environment (26). The auditory association cortex is interconnected with the thalamus. This is a fronto-temporal circuit, which is also connected with the prefrontal cortex to receive unusual auditory stimuli and to enable the elaboration of an integrated auditory memory. Our recordings were made in the temporal region homolateral to the hearing stimulus. In all responsive neonates, except for a neonate who had not increased oxyhemoglobin, $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}_{\text{sum}}]$ increased, and so did ΔCBV , whereas $\Delta[\text{Hb}]$ increased in 61.5% of these neonates, similarly to Sakatani's results. Different patterns in evoked cortical responses are probably due to the different phases of the brain development, which are age-dependent. NIRS findings during brain activation in young adults and healthy subjects demonstrated an increase in local HbO_2 and corpuscular blood volume, with a decrease in Hb (4). PET study demonstrates a greater increase in cerebral blood flow than in oxidative metabolism during somatosensory brain stimulation (27). With the aid of PET and MRI studies, in the newborns with no increase in $\Delta[\text{Hb}]$, glucose consumption rather than oxygen extraction can be suggested (28,29). This result is consistent with Meek's findings in the visual stimulation of neonates, which confirm an increasing CBV with visual stimulation following increases in $[\text{HbO}_2]$ and $[\text{Hb}]$ in most cases, unlike the results of her adult study (7,8). The increase in $\Delta[\text{HbO}_2]$ and ΔCBV after the test prompts several considerations. All responsive newborns had a persistent increase in ΔCBV and did not return to basal ΔCBV values after the stimulation. Electrophysiological data from Alain *et al.* (30) show evidence of a temporal-prefrontal neocortical pathway for the transient storage of auditory stimuli in adults. This fronto-temporal circuit may detect unusual events in the environment, such as

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- our sweep, and justifies the brain work in storing the auditory information after the test. We speculate that there may be a memory in the neonates, or an activation of the temporal-parietal circuit for auditory sensory memory. We used the NIRS method to study a cortical temporal response in sleeping and/or waking neonates. Mean Δ CBV changed from + 0.006 mL/100 g (pretest) to + 0.09 mL/100 g (in-test) and +0.04 mL/100 g (posttest). To date the literature has described an increased Δ CBV on visual stimulation (7, 8) or hypoxic-ischemic brain injury (18, 31–33) or increasing P_{CO_2} (15). Pellicer *et al.* (34) studied NIRS response to an i.v. administration of dexamethasone and found no changes in CBV, but a significant increase in cerebral blood flow. CBV decreases have been observed in relation to the administration of indomethacin *versus* ibuprofen in preterm infants with PDA and in open endotracheal suctionings or during the withdrawal procedure of exchange transfusions (35–37). Our patients were studied only in the phases of auditory stimulus; no frontal or occipital recordings were feasible. No changes in the redox state of cytochrome oxidase were studied. No changes in Cu_A redox state were expected because—in animal models with normal cerebral perfusion at least—large-scale changes in the cytochrome oxidase NIR signal are observed only when there is a severely compromised oxygen supply to the tissues (38). Movement artifacts are a frequent problem in studies on alert children, but we filtered the data to cancel the spikes from the tracing. 31.5% of our neonates did not present any evoked response to the stimulus, the reason why they were arbitrarily considered as nonresponders. However, Münger *et al.* (39) demonstrated that cerebral oxyhemoglobin and total Hb concentrations, measured by light-conveying bundles, positioned in the fronto-temporal and, on the same side, in the parieto-occipital areas, decreased significantly from quiet to active sleep in healthy newborns. Our percentage of unresponsive neonates may be related more to the change of this physiologic sleeping condition than to a disturbed/abnormal auditory evoked response.
- NIRS application is useful for evaluating the temporal region evoked response of the developing brain cortex in neonates, but it is not feasible for screening purposes. When we compare NIRS data with BAEP results, we find no consistency in several cases. Auditory cortical evoked responses show a maturation during the period from 28 wk to 16 yr, reflected by a different waveform morphology; the different age of BAEPs evaluation, and also the time of myelogenetic cycles (40, 41) may in part justify the inconsistency of the test results in the newborns we studied. In conclusion we consider the application of NIRS to auditory function a valid tool for obtaining more information on brain auditory area metabolism and bloodflow changes. These data confirm that NIRS has a valid role in the mapping of the hemodynamic evoked responses and in the study of oxygen metabolism as well as in brain development in newborns.
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