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Neonatal cortical auditory evoked potentials are affected by clinical conditions occurring in early prematurity

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Abstract

Introduction. Cortical auditory evoked potentials may serve as an early indicator of developmental problems in the auditory cortex. The aim of the study is to determine the effect on neonatal cortical auditory processing of clinical conditions occurring in early prematurity.

Methods. 67 preterm infants born at 29 weeks mean gestational age (range 23–34) were recorded at a mean post conception age of 35 weeks, prior to discharge from third level Neonatal Intensive Care Unit. The average of 330 responses to standard 1000 Hz pure tones delivered in a oddball paradigm were recorded at frontal location. Data of 45/67 recruited premature infants were available for analysis. Mean amplitudes calculated from the data points of 30 ms centered on P1 and N2 peaks in the waveforms of each subject were measured. The effect of perinatal clinical factors on cortical auditory evoked responses was evaluated.

Results. The amplitude of P1 component was significantly lower in infants with bronco-pulmonary dysplasia (p=.004) and retinopathy of prematurity (p=.03). The Multivariate Analysis, done to evaluate the relative weight of gestational age and bronco-pulmonary dysplasia and/or retinopathy of prematurity on cortical auditory evoked potentials components, showed an effect of clinical factors on P1 (p=.005) and of gestational age on N2 (p=.02).

Conclusions. Cortical auditory processing appears to be influenced by clinical conditions complicating extremely preterm birth.

1. Introduction

Cortical auditory evoked potentials (CAEPs) are electrical potential changes generated by cortical processing occurring after auditory stimulation. The neonatal overall waveform has no resemblance to that of an adult, and probably reflects different functions and generators undergoing enormous changes in the course of human life (Picton and Taylor, 2007). During the first 20-45 weeks of gestation the development of complex cortical and subcortical networks is modulated by sensory driven development of the talamo-cortical afferents and their connections with the developing cortical plate (Kostovic´ and Jovanov-Milosevic´, 2006). Early studies investigating CAEPs in premature infants showed a typical sequence of waveform changes (Weitzman and Graziani, 1968; Kurtzberg et al., 1984) similar to that evoked using other sensory modalities (Suppiej, 2007). There is emerging evidence that neurophysiological changes may be the functional correlate of early phases of cortical development thus representing a window on the brain at work both in normal and pathological conditions (Kostovic´ and Jovanov-Milosevic´, 2006; Vanhatalo and Kaila, 2006; Suppiej et al., 2011).

The evoked potentials methodology allows non invasive evaluation of brain function at bedside even in preterm neonates admitted to neonatal intensive care units (NICUs). The setting for recording brainstem auditory evoked potentials for neonatal auditory screening protocols (Suppiej et al., 2007) has been used with success to record CAEPs in preterm infants before discharge from NICU (Suppiej et al., 2010).

Longitudinal studies of evoked potentials at different post conception ages have been very important for our understanding of early developmental trajectories, but they don't allow the highlighting of differences in maturation between infants born at different gestational ages. By contrast, cross-sectional studies compare premature infants born at different gestational ages but at the same post conception age. They offer a unique opportunity to evaluate possible developmental differences between early and late prematurity and their relationship to clinical conditions. Indeed, cross sectional studies of CAEPs in preterm infants suggested differences in the ability to detect

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auditory pitch changes between infants born at gestational age higher and lower than 29 weeks (Bisiacchi et al., 2009; Mento et al., 2010). Whether these findings depend on clinical factors characterizing very early prematurity or on extra uterine experience has not yet been explored.

The above issue is pertinent to the research question of whether normal preterm birth does really exist or the underlying cause of preterm parturition is also implicated in subsequent neonatal morbidity and an abnormal neurodevelopment outcome (Ballantyne, 2008).

The objective of the present study is to compare CAEPs recorded at the same post conception age in preterm neonates born at early and late gestational ages, and to correlate CAEP waveform to neonatal clinical conditions. The ultimate goal is to evaluate whether clinical conditions implicated in early and late prematurity affect development of cortical auditory processing.

2. **Methods**

2.1. Subjects

Sixty-seven premature infants (mean gestational age 29 weeks, range 23-34) admitted to our third level NICU, were recruited before discharge at a mean post menstrual age of 35 weeks (range 35 ± 2 gestational weeks). For each patient we collected from clinical records information on gestational age in weeks, determined by the maternal date of the last menstrual period. We also collected the following clinical data selected because of their implications in early preterm birth (Verma et al., 1997; Yoon et al., 1999; Zanardo et al., 2008; Lahra et al., 2009; Moscuzza et al., 2011): bronco-pulmonary dysplasia defined as oxygen dependence at 36 weeks corrected gestational age, retinopathy of prematurity as defined by Fielder et al. 2004 (Fielder et al., 2004), ultrasound evidence of intra-ventricular hemorrhage graded following Volpe et al., 2000 (Volpe, 2000) and of periventricular cystic leukomalacia as defined by De Vries et al.,1992, intrauterine growth restriction defined as estimated foetal weight below the 10th percentile and umbilical artery pulsatility index more than 2 standard deviations (Cosmi et al., 2009), infection including evidence

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of positive blood culture and suspect cases of sepsis with increase of plasma C-reactive protein level.

The infants treated with drugs with central nervous system effect (sedatives) and those with neonatal malformations were not included.

Infants were grouped based on gestational age: born at 23–28 gestational weeks and born at 29–34 gestational weeks.

Out of 67 recruited neonates 22 were excluded from analysis because of failure of neonatal hearing screening or recordings with less than the required artefact-free trials in active sleep state. The final group consisted of 45 newborns (Table 1).

The ethical committee of the hospital approved the study. Parents gave informed consent.

2.2. Procedure

Recordings were performed the day before discharge from NICU when neonates were clinically stable and lying in their cribs between two morning feeds. No sedation was used. Brainstem auditory evoked potentials were recorded before CAEPs for hearing threshold evaluation, using a "Galileo NT" evoked response system (EBNeuro/Florence, Italy) with the method previously described (Suppiej et al., 2007).

The method for CAEP recordings was also previously described (Suppiej et al., 2010). In brief, the stimulation paradigm consisted of a random series of deviant tones at 2000 Hz (occurring with a probability of 10%) embedded among standard tones at 1000 Hz (90%) delivered binaurally via headphones. The tone duration was 100 ms (5 ms rise and 5 ms fall time), the intensity 75 dB nHL. To minimize possible refractoriness effects in premature infants, the inter-stimulus interval was randomized between 1250 and 1850 ms (offset-to-onset).

Recordings were obtained in active sleep: a researcher trained in neonatal behavioural assessment observed the neonate for clinical classification of sleep states, CAEPs recording started as soon as an active sleep state was identified. We excluded trials where indeterminate state, awakening or

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quiet sleep occurred. The sounds were delivered in two blocks of 330 stimuli. Recordings lasted 30-90 minutes including electrode placement and necessary breaks.

2.3. Data acquisition and analysis

EEG was recorded at midline electrode site Fz and referenced to the bilateral linked mastoids, in accordance with the 10–20 international system (Jasper, 1958), using Ag/AgCl electrodes. Only Fz site was used since we previously demonstrated that cortical auditory evoked potentials recorded in active sleep had a fronto-central distribution on the scalp (Suppiej et al., 2010). Furthermore, we needed to minimize handling given the clinical condition of the prematurely born participants. All electrode impedances were less than 10 kΩ and balanced. The ground electrode was placed on the left side of the forehead (Fp1). Vertical eye movements were monitored with a bipolar electro-oculogram.

Data were recorded with an EBNeuro system (EBNeuro/Florence, Italy), amplified and digitized with a sampling frequency of 512 Hz. The band-pass filtering was 0.1–100 Hz, sensitivity 100 µV, analysis time 1 second (including 100 ms pre-stimulus baseline). Trials contaminated by artefacts (caused by eye movements or muscle activity) exceeding 100µV in any channel were automatically rejected.

To improve the definition of waveforms the stored traces were filtered off-line (0.5 Hz high pass and 15 Hz low pass) as it has been shown that this do not interfere with visualization of CAEP responses [23]. The CAEP was analysed in the temporal window from 100 ms before to 900 ms after stimulus onset. Only responses to the standard stimuli were available for analysis because in the average of responses to deviant stimuli, which were 10% of the total block of stimuli, the signal-to-noise ratio was too low. Responses evoked by standard tones showed a positive–negative complex with the positive deflection in the 100–200 ms latency range (P1) and the negative in the 150–250 ms latency range (N2). We measured mean CAEP amplitudes calculated from the data points of 30 ms centered on P1 and N2 peaks in the waveforms of each subject, because it has been

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shown in our previous work to be a reliable method for measurement of CAEP amplitudes in neonatal recordings (Bisiacchi et al., 2009).

2.4. Statistical analysis

A T-test for independent variables was used to compare the continuous normally distributed data; normality was established by applying the Kolmogorov-Smirnov test. Chi-square test and Fisher's Exact test were used for dichotomous variables. In order to analyse the relative effect of gestational age and clinical parameters, we performed a Multivariate Analysis including gestational age and a single clinical parameter, made of the presence of at least one of the clinical data having been shown to have a significant effect on CAEPs on T-test analysis.

The linear correlation between CAEP components was evaluated with the r of Pearson. The significance was set at $p < 0.05$. Bonferroni correction was used for multiple comparisons. All analyses were performed using the Statistical Package STATISTICA 6.0 for Windows (StatSoft, Tulsa, OK).

3. Results

The amplitude of P1 and N2 components of CAEPs in infants with and without bronco-pulmonary dysplasia, retinopathy of prematurity, intra-ventricular hemorrhage, intrauterine growth restriction and infection are summarized in Table 2: it shows a significantly lower amplitude of P1 component in those affected by bronco-pulmonary dysplasia and retinopathy of prematurity.

Furthermore retinopathy of prematurity and bronco-pulmonary dysplasia were significantly more represented in infants born at 23–28 gestational weeks than in those born at 29–34 gestational weeks (bronco-pulmonary dysplasia: X^2=4.55, p=.02; retinopathy of prematurity: X^2=7.30, p=.02). By contrast, intrauterine growth restriction was more frequent in those born at 29–34 gestational weeks, but the difference was not statistically significant.

A lower amplitude of P1 and N2 components was found in infants born at 23–28 gestational weeks compared with those born at 29–34 gestational weeks, but a statistical significance was found only

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for N2 component (t=2.35, p=.02). Mean amplitude and standard deviation of P1 and N2 components of CAEPs in the two groups of infants born at 23–28 and 29–34 gestational weeks are summarized in Table 3.

The Multivariate Analysis, done to evaluate the relative weight of gestational age and clinical factors (bronco-pulmonary dysplasia and/or retinopathy of prematurity) on CAEP components, showed an effect of clinical factors on P1 (F=8.78, p=.005) and of gestational age on N2 (F=5.29, p=.02).

No linear correlation between P1 and N2 was found.

4. Discussion

The present study demonstrated an effect of clinical conditions occurring in early prematurity on neonatal cortical auditory evoked potentials, suggesting the possibility of an interference with development of auditory processing.

CAEP is one of the ontogenically earliest responses and can be recorded as early as 24 weeks of gestational age in infants born prematurely (Weitzman and Graziani, 1968). At that time cortical development is still very immature and afferent fibers are only beginning to be seen in the cortical plate (Krmpotic´-Nemanic´ et al., 1983). In neonates born preterm a broad negativity peaking around 170-280 ms is seen at early gestational ages (Weitzman and Graziani, 1968; Pasman et al., 1991) while, as the premature approaches term, the response changes from surface negative to surface positive. This takes place first at the midline electrodes and, by 1 to 2 months of life, also at temporal electrodes (Kurtzberg et al., 1984). During transition from early prematurity to full term, CAEP waveform is for some weeks dominated by a positive/negative complex that in our recordings at 35 weeks corrected age was characterized by a positive peak at around 150 ms (P1), and a negative peak at around 250 ms (N2).

Cross-sectional studies of CAEP at near term post conception age have already shown differences in developmental trajectory between infants born at very low and extremely low gestational age

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(Bisiacchi et al., 2009; Mento et al., 2010). In the present study we confirmed previous results and, more importantly, we found that clinical conditions occurring in very preterm NICU-admitted neonates were implicated in CAEP waveform differences between very low and extremely low gestational age subjects. Furthermore gestational age and clinical conditions seemed to affect different unrelated components of CAEP waveform, P1 and N2, suggesting that they may represent different underlying generators.

CAEPs are thought to represent the activation of auditory cortical neurons mainly concerned with sensory sound perception and auditory processing. Although functions and generators of the neonatal overall waveform have not yet been clarified, some authors suggest a correspondence between adult and infant CAEP (for a review see Wunderlich and Kone-Wessohn, 2006; Picton and Taylor, 2007). Indeed, intracortical recordings showed that the cortical generator of adult P1 is located in the lateral portion of Heschl's gyrus (Liégeois-Chauvel et al., 2001). By contrast the adult N1 (possible precursor of neonatal N2) is the sum of at least three sub-components, with the most important (N1b) reflecting the activity of highly refractory neurons in the primary auditory cortex (Jacobson et al., 1992).

The most important result of the present study is the finding that there is an effect of clinical factors on CAEP waveform, particularly a P1 mean amplitude reduction in the group of infants affected by bronco-pulmonary dysplasia and retinopathy of prematurity.

The suggestion that a cognitive dysfunction later in life can be predicted by abnormal auditory event related potentials recorded at birth and during development has been an interesting research hypothesis in learning disabilities (Leppanen et al., 1997), autism (Lepistö et al., 2005), cleft palate (Cheour et al., 1999), attention deficit disorder (Potgieter et al., 2003) and Asperger's syndrome (Kujala et al., 2005), as well as in prematurity (Fellman et al., 2004).

Birth at extremely low and very low gestational age (i.e. before 29 weeks gestation) in the majority of cases is due to spontaneous preterm labor and premature rupture of membranes. It is often associated with intrauterine inflammation or chorioamnionitis (McElrath et al., 2008), with the

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proportion of preterm infants exposed to chorioamnionitis increasing with decreasing gestational age to up to 80% below 28 weeks of gestation (Andrews et al., 1995). Research has pointed out that in-uterus inflammation which leads to a fetal inflammatory response, can explain neonatal morbidity, particularly bronco-pulmonary dysplasia and retinopathy of prematurity as well as the more severe stages of intraventricular hemorrhage and of periventricular leucomalacia (Verma et al., 1997; Gomez et al., 1998; Yoon et al., 1999; Zanardo et al., 2008; Suppiej et al., 2009; Moscuzza et al., 2011). Our population of preterm neonates did not include severe intra-ventricular hemorrhage nor cystic periventricular leukomalacia, but interestingly a lower amplitude of P1 component of CAEP was seen in those affected by retinopathy of prematurity and bronco-pulmonary dysplasia.

During the second half of gestation and the neonatal period, the major growing afferents from the thalamus spread within the transient subplate zone, they relocate in the cortical plate, and they form a significant number of functional synapses with both transient and permanent neuronal populations (Kostovic´ and Jovanov-Milosevic´, 2006). It has been shown that high levels of proinflammatory cytokines induce inflammation-mediated impairment of oligodendrocytes (Yoon et al., 1997; Ogunyemi et al., 2003) and secondary neuronal loss and impaired neuronal guidance (Kadhim et al., 2003; Leviton and Gressens, 2007). Furthermore, subplate neurons are selectively vulnerable at their peak in development from 20 to 30 gestational weeks. We would therefore hypothesize that P1 component of CAEP may indicate a disturbance of the above processes of auditory cortical development. The finding by Mikkola et al. (2007) of an abnormal P1 component at 5 years of age in children born preterm further supports the role of CAEP as an early indicator of disturbed developmental trajectory in this population, with a long lasting effect later in life.

While major impairments such as cerebral palsy, sensorineural deficits and epilepsy can usually be predicted by clinical neonatal neurological risk factors, less severe neurodevelopmental outcomes are more difficult to predict (Latal, 2009). Long term follow up of preterm neonates recruited for the present study would be useful to clarify whether failure to develop P1 component of CAEP might be an early indicator of future neurodevelopmental disabilities.

Our study also suggests a selective vulnerability of the N2 component to birth at extremely low gestational ages. Auditory sensory stimulation is known to modulate and shape maturational processes in the auditory areas. It is tempting to hypothesize that auditory overstimulation of the infants in the NICU could affect development of highly refractory neurons in the auditory cortex. Unfortunately, our study was not designed to evaluate this issue further.

In conclusion, our results confirm previously reported differences in CAEP developmental trajectory between infants born at very low and extremely low gestational age. More importantly, they suggest that clinical conditions such as bronco-pulmonary dysplasia and retinopathy of prematurity, typically occurring in NICU-admitted very preterm infants as the result of foetal inflammatory response, may affect cortical auditory processing as indicated by CAEPs.

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FIGURE LEGENDS

Figure 1. CAEP waveforms of neonates with and without bronco-pulmonary dysplasia and/or

retinopathy of prematurity .

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Table 1: Neonatal clinical data

GENDER (Male)	23/45	(51%)
GA ($\leq$ 28)	28/45	(62%)
BPD	7/45	(16%)
ROP	8/45	(18%)
IVH grade I-II	5/45	(11%)
IVH grade III-IV	0/45	(0%)
Cystic PVL	0/45	(0%)
IUGR	14/45	(31%)
Infection	11/45	(24%)

Legend: GA: gestational age; BPD: bronco-pulmonary dysplasia; ROP: retinopathy of prematurity; IVH: intra-ventricular hemorrhage; PVL: periventricular cystic leukomalacia; IUGR: intrauterine growth restriction.
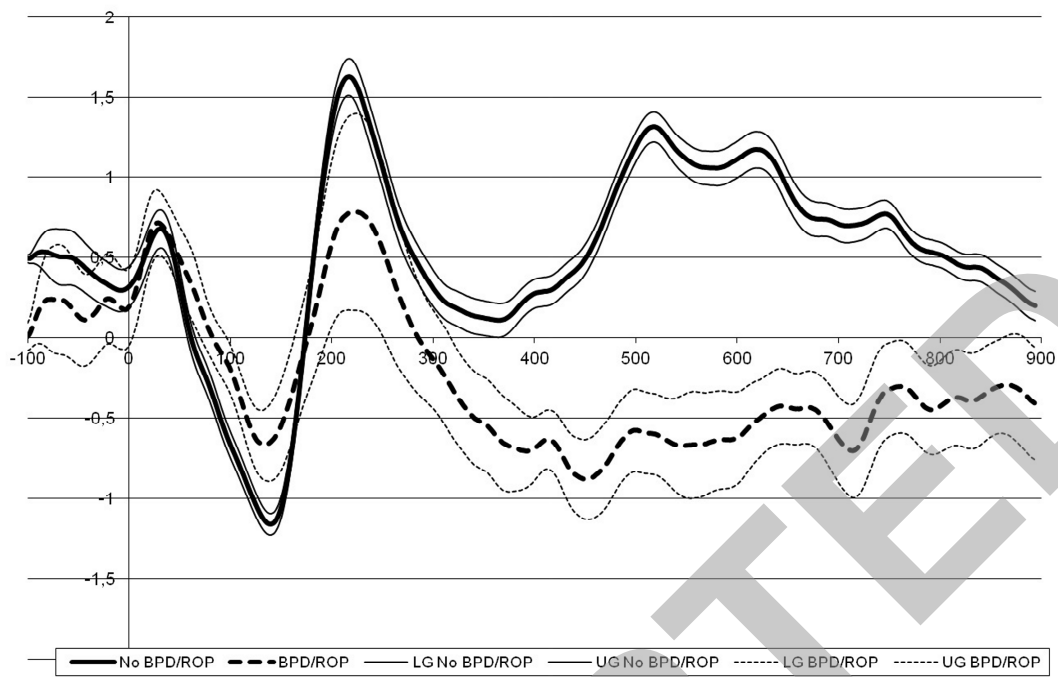
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Table 2: Mean amplitude (M), Standard Deviation (SD) and P-value of T-test analysis of P1 and N2 components of CAEPs in infants with and without bronco-pulmonary dysplasia (BPD), retinopathy of prematurity (ROP), intra-ventricular hemorrhage (IVH), intrauterine growth restriction (IUGR), infection.

Clinical data	N	P1 (M±SD)	*p*	N2(M±SD)	*p*
BPD	YES (N=7)	0.32 ± 0.18	**0.004**	-0.86 ± 1.24	ns
	NO (N=37)	1.14 ± 0.69		-1.53 ± 1.19	
ROP	YES (N=8)	0.50 ± 0.46	**0.03**	-0.59 ± 0.77	ns
	NO (N=37)	1.13 ± 0.69		-1.70 ± 1.32	
IVH	YES (N=38)	0.89 ± 0.75	ns	-0.29 ± 0.66	ns
	NO (N=5)	1.04 ± 0.69		-1.65 ± 1.29	
IUGR	YES (N=14)	1.07 ± 0.77	ns	-1.42 ± 1.12	ns
	NO (N=31)	0.99 ± 0.67		-1.54 ± 1.40	
INFECTION	YES (N=11)	0.87 ± 0.67	ns	-1.40 ± 1.15	ns
	NO (N=29)	1.12 ± 0.84		-1.54 ± 1.80	

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Table 3: Mean amplitude (M), Standard Deviation (SD) and P-value of T-test analysis of P1 and N2 components of CAEPs in the two groups of infants born at 23–28 and 29–34 gestational weeks.

	N	P1 (M±SD)	*p*	N2 (M±SD)	*p*
GA≤ 28	N=20	0.83 ± 0.65		-0.79 ± 0.85	
			ns		**0.02**
GA>28	N=25	1.37± 0.84		-2.12 ± 1.35	

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