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BRAIN ELECTROPHYSIOLOGICAL DEVELOPMENT

IN PREMATURE INFANTS

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INDEX

ABBREVIATIONS

GA: gestational age

PCA: post-conception age

EEG: electroencephalogram

CAEP: cortical auditory evoked potentials

VEP: visual evoked potentials

SEP: somatosensory evoked potentials

ERP: event-related potentials

MMN: mismatch negativity

ELGA: extremely low gestational age

VLGA: very low gestational age

LGA: low gestational age

ABSTRACT

Background. Improvements in postnatal care provided in neonatal intensive care units have resulted in increasing survive percentage of children born at the limits of viability. A large number of premature infants experienced major impairment and/or minor neurodevelopmental disabilities, such as cognitive, psychiatric and motor disorders. The etiology of these developmental deficits still remains not completely understood, but they may be the result of neonatal brain injury as well of interruption of the normal process of brain maturation that occurs during the last trimester of pregnancy, a critical period of prenatal ontogenesis.

Prediction of the outcome of individual preterm infants is difficult. Although a premature infant may be asymptomatic for abnormal clinical signs, he may exhibit subtle alterations in brain activity which often remain unrecognized. A neurophysiologic evaluation of brain activity in the third trimester of gestation would probably be of great benefit for early detection of pathological processes or subclinical alterations. Electroencephalogram and cortical auditory evoked potentials turned out to be simple and useful techniques in evaluation of brain maturation.

Aims. We conducted cross-sectional and longitudinal investigations at early crucial phases of development (35 and 40 weeks post-conception) in order to identify differences in cerebral activity between premature infants born at different gestational ages and full-term neonates, using electroencephalogram (EEG) at rest and cortical auditory evoked potentials (CAEP). We further aimed to correlate the neonatal data with later neurodevelopment.

Methods. The research is divided into three studies: Study 1: EEG spectral activity was recorded at 35 post-conception weeks in 40 premature infants and compared between groups of infants born at different gestational age ("extremely low

gestational age", ELGA: 23–$27^{+6,}$ "very low gestational age", VLGA: 28–31^{+6} and "low gestational age", LGA: 34-35). The results were correlated with behavioral developmental scores obtained at 12 months corrected age from 20 infants. Study 2: a subgroup of 10 infants of Study 1 repeated the EEG recording at 40 post-conception age. EEG spectral activity of this subgroup was compared longitudinally and further the activity recorded at 40 GA were compared with those of a group of 10 full-term infants. Study 3: CAEP were recorded in active sleep at 35 post-conception weeks in response to an auditory stimulation in 36 premature infants and compared between groups of infants born at different gestational age (ELGA, VLGA, LGA). The results were correlated with behavioral developmental scores obtained at 12 months corrected age from 20 infants.

Methodology Study 1 and 2. Electrical brain activity was recorded for 40 minutes on 5 bipolar channels. Data were transformed into the frequency domain using a Fast Fourier Transform algorithm. Frequency spectrum was divided into the following bands: δ (0.5-4 Hz, comprising δ1 0.5-1 Hz and δ2 1-4 Hz), θ (4-8 Hz), α (8-13 Hz) and β (13-20 Hz). Statistical analysis were performed on absolute and relative power values only on central sites (C3-C4, C3-T3, C4-T4).

Methodology Study 3. 1000 Hz (paradigm 1) and 500 Hz (paradigm 2) auditory stimulations were performed on continuous EEG recording. Design consisted of 300 tones for each paradigm. Inter-stimulus interval randomly varied between 600 and 900 ms; 12 monopolar channels were recorded, referenced to the bilateral linked ear lobes. 600 ms epochs were divided for statistical analysis in time windows of 100 ms. Statistical analysis were performed only on central sites (Fz, Cz).

Results. Study 1. On C3-C4, relative spectral power values differed significantly between ELGA and LGA groups. Infants born at lower gestational ages had a higher amount of power in the δ and a lower amount of α and β spectral power. The

preliminary data on those infants attaining 12 months of corrected age showed that higher amount of δ and a lower amount of β and α resulted associated with poor relational skills and personal self autonomies. Study 2. At 40 post-conception age, premature infants showed on C3-C4 a decrease in δ activity and a mild, not significant, increase in higher frequencies; no significant differences in spectral power values were found with full-term neonates. Study 3. In response to 1000 Hz tones no waveforms became evident on Fz in ELGA infants, while LGA presented a wide and slow positive response; the groups differed significantly. VLGA's grand average waveform resembled that of LGA group, but characterized by a high variability. Responses to 500 Hz resulted highly variable and not reliable.

Conclusions. We found early subtle brain electrical alterations in premature infants experiencing different developmental pathways, suggesting a different cortical organization; these differences seem to be associated with later development. The potential of neurophysiological methodologies is to provide a useful indicator of good prognosis or poor developmental outcomes.

RIASSUNTO

Premesse. Gli avanzamenti tecnologici che negli ultimi decenni hanno caratterizzato le cure perinatali e le tecniche di terapia intensiva neonatale hanno permesso la sopravvivenza di una percentuale sempre maggiore di neonati prematuri nati ad età gestazionali sempre più basse, ai limiti della sopravvivenza. Eppure, studi sullo sviluppo a breve e lungo termine hanno dimostrato che molti neonati prematuri riportano esiti maggiori e/o disordini evolutivi minori, come deficit cognitivi e neuropsicologici, disturbi psichiatrici/comportamentali e motori. La causa di tali disordini dello sviluppo rimane poco chiara, ma può essere il risultato di sofferenza cerebrale in epoca neonatale come anche dell'interruzione del normale processo di sviluppo che avviene nel terzo trimestre di gravidanza, un periodo estremamente critico per la maturazione cerebrale.

Predire come sarà lo sviluppo di un neonato prematuro rimane attualmente molto difficile. Infatti, sebbene un neonato possa essere asintomatico per segni clinici indicativi di una condizione patologica in atto, possono essere presenti alterazioni subcliniche del funzionamento cerebrale che spesso non vengono riconosciute. Una valutazione neurofisiologica dell'attività cerebrale nel neonato prematuro può probabilmente essere di grande utilità nel precoce riconoscimento di processi patologici o di alterazioni subcliniche. L'elettroencefalogramma (EEG) e i potenziali evocati uditivi corticali (CAEP) si sono dimostrati tecniche semplici e valide nel valutare la maturazione cerebrale.

Obiettivi dello studio. Abbiamo condotto delle valutazioni neurofisiologiche trasversali e longitudinali in due fasi precoci e cruciali dello sviluppo (35 e 40 settimane postconcezionali) allo scopo di identificare differenze nell'attività elettrica cerebrale fra prematuri nati ad età gestazionali diverse e neonati a termine, usando

EEG a riposo e i CAEP. Tali indagini in epoca neonatale sono state poi correlate con lo sviluppo comportamentale a distanza.

Metodi. La ricerca è stata articolata in tre studi: Studio 1: è stata eseguita l'analisi spettrale dell'EEG registrato a 35 settimane postconcezionali in 40 neonati prematuri; tale attività è stata comparata fra gruppi di neonati nati ad età gestazionali diverse (estremi prematuri, ELGA: 23–27^{+6}, veri prematuri, VLGA: 28–31^{+6} e prematuri, LGA: 34-35). I risultati ottenuti in epoca neonatale sono stati correlati con l'indice di sviluppo comportamentale ottenuto ai 12 mesi di età corretta nei primi 20 bambini che hanno raggiunto tale età. Studio 2: un sottogruppo di 10 neonati dello Studio 1 ha ripetuto la registrazione EEG a 40 settimane postconcezionali; la potenza spettrale ottenuta dalle registrazioni EEG a 35 e 40 settimane postconcezionali è stata cofrontata longitudinalmente; successivamente l'attività spettrale ottenuta alle 40 settimane postconcezionali è stata confrontata con quella di 10 neonati a termine alla nascita. Studio 3: i CAEP sono stati registrati in sonno attivo a 35 settimane postconcezionali in 36 prematuri e comparati fra gruppi di neonati nati ad età gestazionali diverse (ELGA, VLGA, LGA). I risultati sono stati correlati con l'indice di sviluppo comportamentale ottenuto ai 12 mesi di età corretta nei primi 20 bambini che hanno raggiunto quest'età.

Metodologia Studio 1 e 2. L'attività elettrica cerebrale è stata registrata per 40 minuti su 5 canali bipolari. I dati ottenuti sono stati trasformati nel dominio delle frequenze utilizzando una trasformazione Fast Fourier. Lo spettro di frequenza è stato diviso nelle seguenti bande: δ (0.5-4 Hz, composto da δ1 0.5-1 Hz e δ2 1-4 Hz), θ (4-8 Hz), α (8-13 Hz) e β (13-20 Hz). Le analisi statistiche sono state eseguite sui valori di potenza assoluti e relativi ottenute solo dai siti centrali (C3-C4, C3-T3, C4-T4).

Metodologia Studio 3. Durante la registrazione continua dell'EEG i neonati sono stati stimolati con treni di toni a 1000 Hz (paradigma 1) e a 500 Hz (paradigma 2). Il

disegno sperimentale prevedeva 300 toni per ciascun paradigma. L'intervallo inter-stimolo variava in maniera casuale fra 600 e 900 ms; sono stati registrati 12 canali monopolari, riferiti bilateralmente ai lobi degli orecchi. Le epoche di 600 ms sono state divise per l'analisi statistica in finestre temporali di 100 ms. Le analisi statistiche sono state eseguite solo sui siti centrali (Fz, Cz).

Risultati. Studio 1. In C3-C4, i valori di potenza spettrale relativa differivano significativamente fra i gruppi di ELGA e LGA. I neonati nati alle età gestazionali più basse avevano una maggiore potenza relativa in δ e una minore in α e β. La correlazione di questi dati con lo sviluppo comportamentale dei primi bambini che hanno raggiunto i 12 mesi di età corretta ha mostrato come alte percentuali di potenza in δ e basse in β e α fossero associate ad abilità relazionali più povere ed autonomie personali meno mature. Studio 2. A 40 settimane postconcezionali i prematuri hanno mostrato in C3-C4 una riduzione di potenza δ relativa e un lieve, non significativo, aumento di potenza nelle alte frequenze; non sono state trovate differenze significative rispetto i neonati a termine. Studio 3. Nel paradigma a 1000 Hz non è stato possibile rilevare nessuna risposta ai suoni nei neonati ELGA, mentre nei LGA in Fz era evidente una lenta ed ampia onda positiva; la grande media dei due gruppi differiva significativamente in Fz. La grande media dei neonati VLGA assomigliava a quella dei LGA, ma era caratterizzata da un'alta variabilità. Le risposte a toni di 500 Hz sono risultate troppo variabili e non riproducibili.

Conclusioni. Confrontando neonati prematuri che hanno sperimentato linee di sviluppo differenti, abbiamo trovato delle differenze sottili nell'attività elettrica cerebrale che suggeriscono un'alterazione dell'organizzazione corticale. Tali differenze sembrano inoltre associate allo sviluppo comportamentale nel primo anno di vita.

Questi risultati suggeriscono che le tecniche neurofisiologiche possano essere molto utili nella prognosi dei neonati prematuri.

1. THE PREMATURE BIRTH

Premature birth is the birth of a baby of less than 37 weeks gestational age. It develops mostly spontaneously and the cause remains often elusive and unknown, although many factors, such as inflammatory processes, appear to be implicated with the development of spontaneous premature birth. Most studies defined prematurity by birth weight rather than by gestational age, due to uncertainty of obstetric estimation of gestational age and the lack of precision in postnatal gestational age assessment (Hack and Fanaroff, 1999). This choice is, however, problematic, because gestational age is the strongest determinant of biologic maturation and viability (Latal, 2009).

Improvements in postnatal care provided in the delivery rooms and neonatal intensive care units have resulted in reduced mortality and increasing percentage of surviving children born preterm (< 37 weeks) and low birth weight (< 2,500 g); the limits of viability is decreased to 22 weeks gestation, with a surviving percentage of about 10% (Hack and Fanaroff, 1999; Aylward, 2002). In Italy 95% of the infants born > 31 gestational age and/or weighted > 1.000 g survive; the surviving rate of infants born 23-24 is instead between 8 and 25 % ("Manifesto dei diritti del bambino nato prematuro", 2010).

Despite these advancements increasing percentage of surviving children, many unanswered questions remain regarding the outcome of these extremely preterm infants. A large number of these infants experienced major disabilities that include moderate/severe mental retardation, sensorineural hearing loss/blindness, cerebral palsy and epilepsy. The incidence of these major handicaps ranges from 6%-8% in low birth weight babies, to 20%-25% rate in extremely low birth weight babies

(Halsey et al., 1993; Hack et al., 1995; Bennett and Scott, 1997; Bhutta et al., 2002; Smith et al., 2011).

In comparison, major handicaps occur in 5% of full-term infants (Hack et al., 1995; Alyward 2002). Statistic in Italy confirmed International reports, showing major impairments affecting about 25% of surviving 23-24 gestational age children ("Manifesto dei diritti del bambino nato prematuro", 2010).

While the incidence of these major impairments appears to be stable in years, the reduction of mortality in the last decades has paralleled by an increasing recognition of neurodevelopmental disabilities in children at school age, long after the presumed pathogenic process has occurred (Smith et al. 2011; Ment et al. 2009). These high prevalence/low severity dysfunctions include cognitive and neuropsychological deficits, developmental coordination disorder and behavioral and psychiatric disorders (Bhutta et al., 2002; Aylward, 2002; Johnson, 2007). It is estimated that these dysfunctions occur in as many as 30% -70% of very low gestational age and/or birth weight infants (Msall et al., 1991; O'Callaghan et al., 1996; Goyen et al., 1998; Taylor et al., 2000; Smith et al. 2011; Ment et al. 2009). In Italy minor impairments were found in 30-35% of extremely low gestational age infants (Manifesto dei diritti del bambino nato prematuro). Although most outcome reports contain relatively few patients and survival statistics vary (Hoekstra et al., 2004), a meta-analysis of data reported in the literature about outcome after premature birth showed that lower birth weight and gestational age were significantly correlated with a general decrease in cognitive test scores, highlighting the developmental vulnerability of the immature brain (Bhutta et al., 2002).

The etiology of the later neurodevelopmental deficits still remains not completely understood, but they may be a result of neonatal brain injury as well as of interruption of the normal process of brain maturation that occurs during the last

trimester of pregnancy (deRegnier, 2005). It has been speculated that medical and environmental conditions leading to premature birth itself may interfere with brain maturation in a crucial phase (deRegnier, 2005; Suppiej et al., 2011). Last trimester of gestation should be indeed regarded as a critical period of prenatal ontogenesis. It is currently unknown how these processes occur in the case of premature births; however, it is clear that premature infants will have not only quantitative differences in body's physiological systems, but will also have a series of qualitative differences in the structural-functional organization of the brain.

Prediction of the outcome of individual premature infants is difficult: long-term outcome information about extremely low birth infants seems unfavorable compared with more mature premature infants (Saigal et al., 2000; Taylor et al., 2000). However, intact survival with normal cognitive function has been reported (Saigal et al., 1991; La Pine et al., 1995). Multiple studies conducted over the decades have also demonstrated the significant impact of socio-demographic and environmental factors (such as age, sex, race, and socioeconomic status) on cognitive and behavioral development in both premature and full-term infants (Aylward, 1992; Hack et al., 1992; Bhutta et al., 2002). Therefore, by considering outcome after premature birth, gestational age and birth weight must be considered in conjunction with other biomedical and environmental risks (Bhutta et al., 2002; deRegnier, 2005).

Premature infants are a heterogeneous group which may experience multiple risk factors: although the infants born at lower gestational ages and weight and with the most neonatal complications statistically havea greater incidence of developmental problems, also those without severe complications may develop impairments; on the other hand, a number of infants with severe medical complications do well (Beckwith and Parmelee, 1986). Although a premature infant may be asymptomatic for abnormal clinical signs, he may exhibit subtle alterations in brain activity which often

remain unrecognized if not matched with abnormal clinical signs or neuroimaging abnormalities (Perlman, 2002; Larroque et al., 2008).

2. THE ANATOMY OF BRAIN DEVELOPMENT

The third trimester of gestation is a crucial period in the brain development, characterized by two major features: the occurrence of histogenetic events and the laminar arrangement of transient circuitry (Kostovic´, 1990; Penn and Shatz, 1999). The most prominent transient layer on fetal brain histology is the subplate zone which contains waiting thalamocortical afferents and is involved in endogenous activity; it disappears at the end of the first year of postnatal. Post mortem studies have shown that the development of transient embryonic and fetal layer closely correlates with other cellular neurogenetic events (Kostovic´, 1990; Levitt et al., 1997; Rakic et al., 2004; De Graaf-Peters and Hadders-Algra, 2006). The development of circuitry and neuronal connections are indeed to be considered in the perspective of other neurogenetic events such as neuronal proliferation, migration, axon outgrowth, dendritic differentiation and cell death. These structural and functional brain changes support the formation of cerebral pathways (path-finding, target selection and growing into the cortical plate).

The transient fetal zones, neurons and waiting afferents participate in transient patterns of cortical activity and are the major source of the structural plasticity in the developing brain. The different steps of development in the third trimester of gestation are also at the basis of the specific pattern of vulnerability of the premature and term infants.

The third trimester comprised the period between the 20 and 45 weeks' gestation; on the basis of major characteristic of the transients pattern of organization it can be divided into four broadly defined phases: fetal (below 24 post-conception weeks), early preterm (24 - 32 post-conception weeks), late preterm (33-35 post-conception weeks) and neonatal phases (36-45 post-conception weeks).

Fetal phase (< 24 post-conception weeks)

This period is characterised by great production of neurons by the germinative matrix and by the migration of neurons to the cortical subplate. This transient structure is the thickest lamina of this period and its development is driven by thalamocortical afferents, callosal and basal forebrain fibers. In the late fetal phase thalamocortical fibers approach their subplate target in different cortical regions (thalamic afferents are documented in the frontal (Kostovic and Goldman-Rakic, 1983) auditory (Krmpotic´-Nemanic et al., 1983), visual (Kostovic and Rakic, 1984) and somatosensory cortex (Kostovic and Rakic, 1990), where they remain with others afferent fibers "waiting" for a prolonged period prior to their ingrowths into the cortical plate (Kostovic´ and Jovanov-Milosˇevic, 2006).

Early preterm phase (24 – 32 post-conception weeks)

Thalamocortical fibers grow into the cortical plate of the frontal, somatosensory, visual and auditory cortices (Kostovic and Goldman-Rakic, 1983; Krmpotic´-Nemanic et al., 1983; Kostovic and Rakic, 1984, 1990). The role of subplate is crucial in this process: the formation of synapses in the deep cortical plate and the continuation of synaptogenesis in the subplate parallels the coexistence of transient endogenous circuitry and thalamo-cortical permanent circuitry (Penn and Shatz, 1999; Kostovic´ and Judasˇ, 2006). Animal studies have shown that thalamocortical afferents terminate on subplate neurons and other thalamic terminals activate cells in the cortical plate, building the first framework for sensory-driven circuitry (Friauf and Shatz, 1991). These interactions between endogenous circuitry of the subplate zone and thalamic, sensory-driven circuitry (Penn and Shatz, 1999; Kostovic´ and Judasˇ, 2006) coincide with appearance of electrophysiological transients (Graziani et al., 1974; Novak et al.,1989; Hrbek et al., 1973; Vanhatalo and Kaila, 2006) and

behavioral phenomena (De Vries et al., 1982; Kostovic´ et al., 1995); furthermore, at this time is possible to record a clear cortical signal in response to any sensorial stimuli (Graziani et al., 1974; Novak et al.,1989).

The majority of synapses in the cortical plate are located on the dendrites, which suggests that they are probably excitatory in nature. Furthermore, studies by acetylcholinesterase (AChE) histochemistry in the human brain show a transient modular distribution and intracortical elaboration of thalamocortical fiber branches, which coincides with incipient areal differentiation (Kostovic and Goldman-Rakic, 1983; Krmpotic´-Nemanic et al., 1983; Kostovic´, 1990). Gradually the elaboration of synapses and growing of dendrites will change the vertical organization of the cortical plate into a radial organization. Ventricular and subventricular zones will became thinner due to the decreased proliferative activity.

Late preterm phase (33 – 35 post-conception weeks)

Subplate zone starts to decline in parallel with the ingrowth of the callosal and long cortico-cortical pathways. Major afferent subcortico-cortical and efferent cortico-subcortical pathways have completed their growth, selected their targets and established synapses. The maturation in the organization of the cortical pathways brings to a better connection between the periphery and the cortex (Kostovic´ and Jovanov-Milos˘evic´, 2006). The afferent fibers build up the corona radiata and adapt their course to the process of gyration. The intracortical circuitry develops with the first evidence of intracortical differentiation (Penn and Schatz, 1999): the major histogenetic event of this period is the appearance of six layers in neocortex (Brodmann, 1909). The cortical layers remain immature, as result of an incomplete development of dendritic trees. This period is characterized also by an intensive differentiation of dendrites of pyramidal neurons. In comparison to postnatal ages,

there are very few spines on dendrites of pyramidal neurons in premature infants (Mrzljak et al., 1988; Mrzljak et al., 1990; Mrzljak et al., 1992). As dendritic spines in adults represent the most common postsynaptic element with a presumably excitatory function, their paucity in late preterm suggests a functional immaturity of cortical neurons.

At this time, the substrate for interaction between cortical areas and the two hemispheres is incomplete primarily because cortico-cortical pathways are still growing. The influence of the sensorial stimulation in this phase still remains not well understood. Data from animal studies show that the stimulation does not affect the number of synapse since after birth, suggesting an endogenous, programmed control of this process before birth; however, it produces changes in the laminar distribution of synapses, indicating a structural plasticity (Bourgeois et al., 1989).

Also the organization of the white matter undergoes changes: the tangential fetal fiber-architectonic stratification transforms completely into a corona radiata system, the centrum semiovale is formed and the gyral white matter becomes visible (Judasˇet al., 2005).

The neonatal period (36- 44 post-conception weeks)

Axonal arborization, dendrites, spines and synapses within the cortical plate layers develop rapidly (Kostovic´ et al., 1995; Mrzljak et al., 1988). No further growth of long afferents and cortico-cortical pathways and corpus callosum's fibers along the interhemispheric pathways trajectory occur, but the retraction of exuberant axons begins. In parallel to cessation of thalamo-cortical growth and early long cortico-cortical callosal fibers, short cortico-cortical fibers develop during several postnatal weeks. The maturation of thalamo-cortical circuitry and interaction with sensory input shapes fine connectivity within the cortical columns (Penn and Shatz, 1999),

underlining the great importance of the sensory-driven cortical activity in neonates during critical periods of development.

3. NEUROPHYSIOLOGY

A neurophysiologic evaluation of brain activity in the third trimester of gestation would probably be of great benefit for early detection of pathological processes or subclinical alterations. In fact, the development of cerebral connections cannot be studied directly, but clinical neurophysiology can give important information about normal and abnormal development of brain function. It has proved to be successful in early assessment of the brain maturation (West et al., 2006) and prediction of later outcome in preterm infants with intraventricular hemorrhage or white matter disease (Hellstro¨m-Westas et al., 2001; 2005; 2006). This could help in early prediction of long-term outcome and facilitate early counseling of parents.

The neurophysiological techniques applied to the study of brain comprise the electroencephalogram (EEG) and the evoked potentials (EPs). These techniques have the advantage over neuroimaging instruments of being "functional" and of providing more accurate temporal information (Mrzljak et al., 1988; Vanhatalo and Lauronen, 2006). Clinical neurophysiology can give important information about normal and abnormal development of brain function, providing insight into brain mechanisms underlying brain maturation and being, at present regarded as possible markers of brain damage during the early phases of development (Cheour et al., 2000).

EEG and EPs can be recorded at bedside in neonatal intensive care units, even in the incubator, in a short time with minimal handling and can be regarded as a non invasive technique even in infants born prematurely (Suppiej, 2007).

3.1 The electroencephalographic activity

The electrical activity recorded from the scalp represents the synchronous activity of neurons arranged perpendicular to the surface of the cerebral cortex. More

specifically, EEG measures voltage fluctuations resulting from ionic current flows within the neurons of the brain. At rest, the activity is characterized by rhythmic oscillations, generated by synchronized synaptic activity of large populations of neurons in thalamo-cortical and cortico-cortical networks. These rhythmic oscillations reflect regular changes in electric potential, the EEG rhythms, which added together produce the so called "background activity". Under stimulation, impulse activity of selected neurons linked to information processing are superimposed to EEG rhythms, and they may be extracted from the ongoing EEG by several methodologies (ERPs, ERS/ERD, etc…).

Common to neurology and sleep studies, EEG background is evaluated qualitatively by visually inspection. It needs a specialist evaluation, especially in pediatric population. The visual inspection of EEG highlights abnormal background activity (e.g. slowing, burst suppression), paroxysmal features and activity; however, this kind of inspection is subjective and doesn't allow to quantify the abnormality or to individuate more subtle alterations, such as lower power amount of any rhythms in EEG spectra contents.

Several mathematical algorithms have developed for quantitative analysis of the EEG. Frequency or spectral analysis involves selection of elementary shapes or frequencies (waveforms) which are added together like weights on a scale until their total matches the pattern under investigated. The height or intensity of a waveform and its amplitude are computed in microvolt for each frequency.

The most popular technique is the Fast Fourier Transformation analysis, a very accurate spectral analysis methodology that decomposes the signal into sinusoidal harmonics. Stability of a signal across time (stationarity) is a prerequisite for accurate Fast Fourier Transformation and a signal is often segmented into short time intervals of like signals to increase its stability. When two or more signals are compared, the

stationarity of phase and amplitude difference (coherence and comodulation, respectively, Sterman and Kaiser, 2001), as well as spatial topography, come into play. The Wavelet Transformation is similar to the Fast Fourier Transformation (or much more to the windowed Fast Fourier Transformation) with a different merit function: in the Fast Fourier Transformation signals are represented as a sum of sinusoids and only localized in frequency, while Wavelets are localized in both time and frequency. This kind of analysis is however actually very little used in neonatal electroencephalographic studies.

3.1.1 EEG rhythms

When Fast Fourier Transformation or Wavelet Transforms are applied to EEG recordings containing rhythms, these rhythms appear at the corresponding spectra in a form of peaks. The EEG rhythms are conventionally divided into the following types: direct current (DC) shifts, decosecond oscillations and slow waves, δ, θ, α, and β EEG rhythms. The significance and the characteristic of these rhythms have been extensively studied in adults and are briefly described (see Kropotov, 2009).

Direct current (DC) shifts, decosecond oscillations, and slow waves (< 1 Hz). The frequency range below 0.1 Hz corresponds to so-called deco-second (with periods of tens of seconds) oscillations. Infra-slow potentials appear to be associated with slow metabolic processes. Slow waves are in the range of 0.1–1 Hz and are present in EEG during all states from the deep sleep to the state of focused attention. They dominate EEG recording in deep sleep - giving it the name "slow wave sleep" – and they are characterized by rhythmic cycles of cortical membrane depolarization (so-called UP states) following by hyperpolarization (so-called DOWN) states. UP states are associated with the increase of discharge rate of a group of cortical neurons

while DOWN states are associated with the decrease of neuronal spiking (Kropotov, 2009).

Delta (δ, range 1–4 Hz). Studies from animal models and adults show the existence of two types of δ oscillations EEG: the first one has a cortical origin, while the second one is generated in the thalamus. The neuronal mechanisms of the cortically generated δ rhythm are unknown. In contrast, the neuronal mechanisms generated in the thalamus are very well known. Δ rhythm can be generated in a single thalamo-cortical cell by interplay of two ion currents (and consequently two types of ion channels) of the thalamic neurons projecting to the corresponding cortical areas. It should be stressed here that the same circuit is involved in generation of spindles (around 13 Hz periodic activity) during sleep and α rhythms (around 10 Hz periodic activity) during wakefulness. A rhythms appear when thalamo-cortical neurons are relative depolarized, sleep spindles appear when these cells are relatively hyperpolarized and δ rhythms emerge at the deepest level of hyperpolarization of thalamo-cortical neurons. Depolarization state of the neuron is usually found in the state of wakefulness; hyperpolarization state is produced during deep sleep due to the suppression of inputs from ascending activating system of the brainstem as well as due to suppression of inputs from the other brain systems (Kropotov, 2009).

Theta (θ, range 4 - 8 Hz). Classically, activities in this frequency range are recorded as trains of rhythmic waves at a frequency of 6–7 Hz with maximum amplitude around the frontal midline, the so called "frontal midline theta rhythm". This frontal midline theta rhythm was often associated to hippocampal θ rhythms, which is thought to be involved in memory encoding and retrieval. It was suggested that the hippocampus functions are those to join together all various and spatially distributed representations of an episode (Klimesch, 1999). Bursts of brief not synchronized

(time locked) cycles in θ band was also revealed by ERD/ERS method during cognitive tasks, as mathematical operations (Kropotov, 2009).

The frontal midline theta shows individual differences and was related to certain personality traits: the amount of θ negatively correlates indeed with scores in the anxiety scale (Inanaga, 1998). Furthermore, θ activity is enhanced in meditation and hypnosis (Crawford, 1994; Takahashi et al., 2005).

Alpha (α, range 8 – 12 Hz). Human brain exhibits several types of distinct rhythmic electrical activity in the α frequency band (8–13 Hz). Three main types of α rhythms were recognized: the posterior α rhythms, recorded at occipital or occipital-parietal areas, the Rolandic or mu-rhythm, recorded over the sensory-motor strip, and midtemporal rhythm which in normal conditions can be recorded only in magneto-encephalogram. These oscillations are driven by rhythmic activity from thalamic nuclei: each rhythm having an origin in a corresponding thalamic nucleus. The frequency of the occipital α rhythm slightly changes with age reaching its maximum at the age of 15–20 and thus declining; due to this decline with age it has often been suggested that the frequency of α activity might reflect cognitive functions of the brain (Posthuma et al., 2001). Posterior α rhythm appears during relaxed wakefulness in eyes closed condition and is suppressed in response to visual stimulation; Rolandic rhythms respond by desynchronization (decrease of amplitude) to actual or imaginary actions. The power of α activity is inversely correlated to metabolic function of the corresponding cortical area giving rise to a functional explanation of α rhythms as idling rhythms of the cortex (Feige et al., 2005).

Beta (β, > 13 Hz). There are several types of rhythms in the β band and it is conventionally divided into the following sub-bands: low β – from 13 to 20 Hz, high β – from 21 to 30 Hz, and γ activity – from 31 Hz (see below). Although not well localized, frequencies in β range are more often recorded on frontal or central areas

when compared to posterior regions of the cortex. On central sides, β rhythm appears in motor-related tasks (Hari and Salmelin, 1997), while on frontal sides β rhythm emerge in cognitive tasks related to stimulus assessment and decision making (Kropotov, 2009). There is a close relationship between EEG power in β band and metabolic activity in the corresponding cortical area of the human brain (Cook et al., 1998).

B rhythm was strongly associated to networks of inhibitory interneurons both theoretically and experimentally (Porjesz et al., 2005). During signal processing inhibitory neurons start firing to suppress the strong activation; this inhibition occurs in cycles and each cycle is a wave of β activity recorded at the scalp. It is thought that β activity acts as a reset operation that clears all sequences of strong activation in neuronal networks and that enables the networks to process information again and again (Kropotov, 2009). The involvement of inhibitory neurons is also supported by the sensitivity of β rhythm to GABAergic agonists – pharmaceuticals that mimic the action of GABA, the main inhibitory mediator in the central nervous system. GABA agonists, such as barbiturates and benzodiazepines, increase indeed the power of high frequency bands.

Gamma (γ, > 30 Hz). Γ rhythm is difficult to record due to their low energy. In animal experiments the synchrony between neuronal elements at 40 Hz has been proposed as a special mechanism of neural cooperation, called "temporal binding". This temporal coordination in spiking of spatially distributed neurons coded different features of the same image is needed to glue together representations of these different features of the image into a single percept (Tallon-Baudry et al., 1999; 2005). Recently, it was speculated that neuronal synchrony may be also critical for conscious processing (see, e.g., Engel and Singer, 2001).

In adults EEG, the dominant frequency recorded is in the α range, typically at 10 Hz. The peak frequency changes considerably during development, steadily increasing from around 6 Hz in the first years of life (Katada et al., 1981; Koroleva et al., 2002). Although a dominant value within the α range is typically reached at approximately 10 years of age (Katada et al., 1981; Lindsley, 1939), this continues to mature during adolescence (Marcuse et al., 2008; Peters_n and Eeg-Olofsson, 1971).

Overall, within the first years of life, there was a rise in spectral power (Sankupellay, 2011; Jenni 2003), with a decrease in lower frequencies and a corresponding increase in higher frequencies. These changes are probably related to structural brain development, potentially reflecting the increasing myelination of brain white matter observed on MR images (Paus et al., 2001). However, the frequency composition of the EEG power spectra continues to change throughout childhood and adolescence, reflecting the increase in dominant frequency. The changes begin over posterior brain regions, where they are also wider (Clarke, Barry, McCarthy & Selikowitz, 2001; Gasser et al., 1988). Over central and frontal areas the maturation is slower and occurs later in development (Gasser et al., 1982; Katada et al., 1981).

Very little is known about neurophysiological development at earlier phase of development, as brain is very immature and its functionality reduced.

4. MATURATION OF EEG ACTIVITY IN PREMATURE INFANTS (23-40 GA)

4.1 Maturation of EEG by visual inspection

The EEG of the premature infant reflects the immaturity of fetal brain. The immature background electrical activity is characterized by discontinuity, labiality and fragmentation. The greater the prematurity, the more marked these EEG aspects are (Suppiej, 2007; Vecchierini et al., 2007). During development the discontinuity gradually decreases providing the most visually striking aspect of EEG maturation. Although normative EEG data for early prematurity are only emerging, normal EEG characteristics and their maturational patterns in premature infants are well established (Vecchierini et al., 2007). The correlation of research on early phases of structural brain development with ontogenesis of EEG is a crucial step in the knowledge of functional brain development (Suppiej et al., 2011).

Fetal phase (< 24 post-conception weeks). For infants born below 24 weeks gestation survival rate is very low and so is the possibility to record the EEG. Engel, 1964, has recorded the EEG of a 19-week-old fetus, detecting initially activity in the 9 to 10/sec range, flattened gradually during the recording. In this period the thalamo-cortical fibers are still waiting in the subplate, thus explaining the lack of EEG activity (Engel, 1964).

Early preterm phase (24 – 32 post-conception weeks). At this time the EEG activity is characterized by a discontinuous pattern consisting in high amplitude synchronized burst of physiological rhythms alternating with periods of low voltage activity (amplitude < 10 μV) of duration corresponding to gestational age (Vecchierini et al., 2007). The EEG inactivity reflects the immaturity of the cortex and its

connections, whereas the bursts activity, endogenous self-organizing figures, probably reflects thalamic subcortical impulses (Scher, 1996), with a probably crucial role in the maturation of the thalamo-cortical connections (Penn and Shatz, 1999; Grubb and Thompson, 2004; Vanhatalo and Kaila, 2006; Price et al., 2006). The increasing activity of the cortical circuitry parallels the decrease of discontinuity. In this phase transient EEG phenomena can be related to the coexistence of transient endogenous circuitry of the subplate and permanent sensory-driven circuitry. With maturation these EEG transients such as centro-occipital delta brushes and temporal saw tooth show a focal distribution over sensory areas (Scher et al., 1994). With development of the cortical circuitry higher frequencies gradually replace slow activity (Vanhatalo et al., 2002). Finally, in this period waking and sleeping states cannot be recognized in EEG, although a cyclic organization of the EEG activity is emerging (Kuhle et al., 2001). Using the direct current EEG (DC-EEG), methodology that allows to avoid the high-pass filter of the conventional EEG, it has been demonstrated that the most prominent spontaneous EEG activity of premature infants consists of very slow, large amplitude transients (the so called "SAT"), possible electrical correlates of cortical development (Vanhatalo et al., 2002).

Late preterm phase (31 – 40 post-conception weeks). The brain maturation provides the neurophysiological basis for a more continuous EEG pattern. At 34 post-conception weeks the EEG activity reaches a continuity of about 80%, and by 35 post-conception weeks the differentiation between quiet and active sleep becomes more evident, with discontinuity observed only during quiet sleep (in the co called "tracé alternant" pattern) (Niedermeyer et al., 2004). The continuous background activity depends on maturation of thalamo-cortical loops, that can arise only after the thalamo-cortical fibers have established their final connections at specific cortical layers (near term age) (Vanhatalo and Kaila, 2006). The maturation of EEG transients

characterizes this period; the temporal sawtooth disappears in active sleep at 32 post-conception weeks and in quiet sleep at 33-34 post-conception weeks; by contrast, delta brush activity decreases in amplitude and frequency and has its full expression between 32 and 34 post-conception weeks. Frontal transients appear at 34 post-conception weeks and become mature at 36 post-conception weeks in quiet sleep (André et al., 2010). From 34 post-conception weeks the EEG activity become synchronous between hemispheres, thanks to development of the corpus callosum. At about 36-38 weeks, the premature babies show EEG features similar to those of full-term neonates. Delta brush activity begins to decline and then disappears (Monod et al., 1983). Wakefulness, active and quiet sleep show three distinctive EEG patterns. From full-term into infancy the maturation of intracortical interneuronal circuits and of inhibitory neurotransmission allows the cortical networks to become able to generate robust higher frequency activity (β and γ), believed to be essential for many cognitive functions (Buzsaki and Draguhn, 2004; Palva et al., 2005; Steriade, 2006).

4.2 Maturation of EEG by quantitative analysis

Technical consideration

A discussion about the spectral content of EEG of premature infants may not go beyond the consideration of the discontinuity of background EEG and of the slow maturational trend to a more continuous pattern, strongly correlated with emerging differentiation of behavioral states. It has shown changes in continuity correlated with changes in the relative power of the δ band; data about the other measurements of the EEG spectrum (θ, α, β frequencies) are contrasting (Victor 2005; Schumacher et al., 2011).

Another challenge in the consideration of the spectral content of premature EEG is the large use of the Fast Fourier Transformation analysis in the literature. Fast Fourier

Transformation specifically requires the stationarity of signals (one of the basic assumptions of Fast Fourier Transformation is that the EEG signal is stationary during a window of 2 seconds), which is impossible to obtain when analyzing long-time epochs of EEG recorded from premature infants because of the discontinuity of the background activity. This problem is particularly challenging for highest frequencies (> 13 Hz), given their relative lower amount on the whole recording. The problem is partially solved by using shorter segments (< 2 seconds) and by implementing overlapping. By calculating robust EEG parameters based on a big amount of assembled data, the effects of non-stationarity in the EEG recordings are minimized. However, δ frequency band remains the spectral parameters at higher repeatability in premature infants, thus accounting almost in part for the partly incongruent results focusing on higher frequencies in the literature. These data point δ spectral power as a potentially useful measurement for EEG analysis in premature infants when the signal is predominantly made up of lower frequencies (Victor et al., 2005; Schumacher et al., 2011).

4.2.1 Review of existing Literature about spectral development in premature infants

Among neurophysiologic techniques, quantitative analysis of the EEG activity – and spectral analysis as the most known and used - has revealed to be a very useful tool in brain monitoring and in assessing cerebral maturation in premature infants (Niermarkt et al., 2011; Victor et al., 2005; West et al., 2006). Spectral analysis of EEG reflects the changes showed by visual inspection.

The interest for the EEG spectral content of premature infants arose several decades ago, in the early '70s-'80s. Afterwards, the advancements in technology of the last years gave new energy to this kind of research.

Despite the numerous studies in the literature, shared normative data about spectral power in premature infants at different post-conception weeks are missing (but see Victor et al., 2005). Furthermore, results of studies are difficult to compare due to differences in methodology: the studied population (e.g., healthy *vs* ill premature infants), the used montages (e.g., bipolar *vs* monopolar, different location sites), the used recordings and analysis parameters, the recordings duration, the behavioral state at the time of recordings, post-conception age at recording, etc…Finally, studies investigating electrical brain activity in premature infants using spectral analysis have different aims.

Consequently, data available in the literature about EEG spectral analysis of premature infants were briefly reviewed and categorized. Studies may be divided into three main categories:

1) studies investigating the development of the electrical brain activity longitudinally during the preterm phase (23 – 40 gestational age);
2) cross-sectional studies comparing premature infants at term corrected age to full-term healthy infants;
3) studies investigating the effects of some pathological conditions on EEG spectral content, in order to identify a useful screening tools.

The following paragraphs illustrate results coming from longitudinal and cross-sectional studies; the third category of studies – with more applicative and clinical purposes – goes beyond the scope of this thesis.

1) Longitudinal studies about early spectral development in premature infants

Immature brain is characterized by a superiority of frequencies in the lower range. In the early preterm phase the lower frequencies (δ band) represent up to 90% of all frequencies recorded, with differences between studies ranging from 65% up to 93%

of all frequencies recorded; the differences are probably due to the large variability of the recordings parameters (the available studies investigating maturation of the EEG spectral power in premature infants are reported in Table 4.1). This high percentage of δ frequencies reflects the awesome presence of high-amplitude slow waves, which appears however to be underestimated by the use of conventional EEG: DC-EEG recordings show that spontaneous premature EEG activity mainly consisted of very slow, large-amplitude transients of < 0.5 Hz (Vanhatalo et al., 2002).

Slow activity is thought to play a major role in the functional and structural shaping of neuronal circuitries in immature brain tissue (Penn and Shatz, 1999; Garaschuk et al., 2001); for the generation of a robust high frequency activity the maturation of intra-cortical inter-neuronal circuits and of inhibitory neurotransmission is required (Buzsaki and Draguhn, 2004; Steriade, 2006; Vanhatalo and Kaila, 2006). Θ, α and β activities are at this time relatively minor components of electroencephalographic activities (Parmalee et al., 1968; Watanabe, 1992; Lombroso, 1985).

Θ relative power amounts from 6 to 12% of the entire recording, α ranges between 3 and8% and β 3 amounts to 6%. Despite the underrepresentation of these frequencies in the EEG of premature infants, specific waveforms in these frequency bands are known as a marker of electroencephalographic maturation – e.g. temporal sharp theta wave -(Watanabe, 1992), therefore, objective evaluation of θ, α, and β spectral activities will be useful to evaluate brain maturation in preterm infants. High-voltage rhythmic temporal theta bursts frequently appear between 27-28 and 33 weeks post-conception age and diminish progressively later (Hughes et al., 1987; Vanhatalo and Kaila, 2006; André et al., 2010). Specific features with a well know localization and timing are considered physiological (Scher, 1997). These bursts of activity were reflected on spectral power as a larger amount of spectral power in their range of frequency.

Although the illustrated spectral content characterizes the EEG of premature infants until they reach term age, subtle changes from 24 to 40 post-conception weeks occur: along with the increase of the post-conception age gradual changes in the EEG spectral power content were shown, as effect of the increase in the dominant frequency and the progressive shift from lower to higher frequencies. It was shown that amplitude of delta waves decreases according to post-conception age (Okumura et al., 2003; Vanhatalo et al., 2002; Bell et al., 1991). Persistently high amount of δ activity may reflect alteration of the normal process of shaping cortical circuitry. It was demonstrated that the persistence of slow delta waves after 33-34 weeks of gestation was related to adverse outcomes (Vecchierini et al., 2007). Conversely to δ activity, data about spectral amount of θ, α and β are not always in agreement. The global amount of absolute power of these frequencies is low, accounting in part for the inconsistent data in the literature (but see "technical considerations"). Many studies found a positive correlation between high frequency power and post-conception age, while others did notfind differences in higher frequency spectra content along with the increase of post-conception age or, sometimes, lower absolute and relative power (Victor et al., 2005; Schumacher et al., 2011). The differences between results may be due to different methodologies, particularly to differences in recording time and in technical and analyzing procedures, generally implemented and focused on other parameters (e.g. δ parameters) and not always adequate for these features.

Literature indicates 36 post-conception age as an important pivotal time around which specific spectral EEG activities have unique maturational trends: total EEG spectral power decreases in value up to post-conception term age, while δ spectral frequency decreases up to 36 weeks post-conception. On the other hand, spectral θ power increases consistently only after 36 weeks post-conception. It has been

reported that spectral α and β powers increase before 36 weeks post-conception and changes in direction with decreasing values after 36 weeks post-conception up to post-conception term ages (Scher et al., 1995).

Table 4.1 shows the details and the results of longitudinal studies investigating the brain development in premature infants using quantitative analysis of the EEG.

Table 4.1 Longitudinal investigations of brain development in premature infants using quantitative analysis of the EEG

Authors	GA	PCA	Behav state	montage	Analysis	Main results
Scher et al., 1995	56 born ≤ 32 GA	28-43	entire cycle	11 elec	sleep architecture, continuity, EEG spectral (FFT), phasic and autonomic measure	variables of brain maturation before 36 PCA: α in QS, total power, arousal in AS, %discontinuity after 36 PCA: θ, β in AS and α in QS were most
Scher et al., 1997	55 born ≤ 32 GA 45 fullterm	at birth	3 h, entire cycle	12 bip	spectral analysis (FFT)	lower spectral energies during sleep in specific regions and asymmetries in preterm group.
Eiselt et al., 1997	7 born < 32 GA 6 fullterm	day 3, 8	sleep	7 bip/ monop (linked ears)	spectral analysis (Hilbert transformation)	higher mean spectral power for the first half of burst than for the second in >2,8-14,8 Hz, most in premature
Holthausen et al., 2000	94 born 24-42GA	28-112	1-6 h, QS and AS	T3-C3, T4-C4	spectral analysis	establishing clinically relevant age dysmaturity scores
Vanhatalo et al., 2002	6 born 33-37GA	33-37	sleep	4-8 electrodes (ref mastoids)	direct current (DC) EEG	presence of slow δ activity is not well recognized in conventional EEG
Okumura et al., 2003	10 born ≤ 34 GA 8o 30??)	within 72h 29-30 31-32 33-34	entire cycle	8 monop (bilateral ears)	spectral analysis (FFT)	Reduction power in 0,53-1 Hz with PCA in all leads and in 1-4 Hz in occipital
Inder et al., 2003	59 born 23-31 GA M weight 1080±389 g	pre-discharge	24-106 h	C3, P3, C4, P4	EEG intensity and amplitude, SEF	VLBW with increasing white matter injury lower SEF, no ≠ for conventional EEG
Iovleva et al., 2004	11 born 38-41GA 12 born 34-36 GA 9 born 30-33 GA	19 days (34-38) 30 days (30-33)				> GA > power in δ,θ,α. Higher coherence in δ; < GA higher coherence in α and θ
Victor et al., 2005a	53 born ≤ 30 GA	each first 4day	75 min, entire cycle	6 monop (ref. Cz)	spectral analyses (FFT) and manual measure of IBI	over 4day increase in relative power of δ and decrease of IBI
West et al., 2005	63 born < 32 GA	first week	sleep	C3, P3, C4, P4	total EEG intensity in 2-20 Hz, SEF	Quantitative measures of EEG continuity increased over first week
Okumura et al., 2006	10 born ≤ 36 GA	29-30 31-32 33-34	entire cycle	8 monop (ref bil ears)	spectral analysis (FFT)	reduction in amplitude with age only in θ1 and θ2

de la Cruez et al., 2007	21 preterm and fullterm	32-37 37-42 42-47	QS, AS	8 average ref	coherence analysis and average linear (AVL), average nonlinear (AVN)	In θ, AVL > with age for centro-temp during QS. In β, AVL > during QS and < during AS with age. β AVL was greater in AS than in QS in P but the reverse in older T. AVN decreased with age during QS.
Tolonen et al., 2007	16 preterm and fullterm	32-46	Postpran dial sleep	DC 4-8 elect., ref ipsil masto	Spectral analysis (wavelets, root mean square)	Low frequency < and high frequency > with age (θ-α) both in continuous and discontinuous EEG. SAT and iSAT remain distinct throughout development
Batuev et al., 2008	60 born 30-32 GA 33-34 GA 35-36 GA 37-38 GA 39-40 GA 41-42 GA	first month	drowsy after fed	10 leads	Power spectra, indexes, and coherence functions	The δ power max in group 5. θ in 3. θ index > in 2,3,4. α in 2. α index in 1,2,3. β1 e β2 indexes in 1,2,4. The > number of coherence in δ,θ,α in6. Non-linear GA dependence
Schumacher et al., 2011	48 born 24-27 GA 28-30^{+6} GA	within 12 h	M=67.9 h, entire cycle	Fp1,P3, O1, T3, Fp2, P4 O2, T4, bipol	spectral analysis (FFT)	Long-term EEG activity depends on GA but the early changes (day 1-3) are independent of GA
Niermarkt et al., 2011	18 born < 32 GA	27+4 to 36+3 weekly	4 h, entire cycle	5 bipol	spectral analysis (FFT)	maturational changes > centrotemporal sites. < abs power δ 1-2,θ; < rel power δ1, > rel power β,α.

2) Cross-sectional studies comparing premature infants at term corrected age and full-term healthy neonates

Despite the numerous studies assessing the typical development of "healthy" premature infants, by comparing full-term infants with "healthy" premature infants attaining term age, the premature infants result in many differences: a delay in maturation of some characteristics of the EEG, but also an advance in some others, probably due to adaption to extrauterine life (Scher et al., 1994). It has often been reported that relative spectral values were lower in the premature neonates for θ, α and β frequency ranges, whereas δ spectral values remained unchanged or increased compared to full-term (Scher et al., 1994; Niermarkt et al., 2011).

These differences are supported by data coming from other physiologic measures (Scher et al., 1992): premature infants seem to have a longer sleep cycle length (e.g. 70

vs 50 minutes), more abundant tracé-alternant quiet sleep (e.g. 32% *vs* 28%) and a less abundant low-voltage irregular active sleep (e.g. 12% *vs* 17%). Fewer arousals and body movements during quiet sleep and REM sleep were also noted (Scher et al., 1994).

Table 4.2 shows the details and the results of the cross-sectional studies investigating the differences between premature infants at term corrected age and full-term neonates using quantitative analysis of the EEG.

Table 4.2 Cross-sectional investigations of differences between premature infants at term corrected age and full-term neonates

Authors	GA	PCA	Behav State	montage	Analysis	Main results
Scher et al., 1996	19 born ≤ 32 GA 19 fullterm	at birth + at term	3 h, entire cycle	11 elec.	FFT, EEG-sleep	lower Bayley scores associated to higher spectral correlations, lower spectral energies in β, fewer arousal/min, lower REM/min, shorter sleep latencies from awake to AS, lower socioeconom.
Scher et al., 1997	55 born ≤ 32 GA 45 fullterm	at birth	3 h, entire cycle	12 bip	FFT	lower spectral energies during sleep in specific regions and asymmetries in preterm group.
Holthausen et al., 2000	94 born 24-42 GA	28-112	1-6 h QS, AS	T3-C3 T4-C4	FFT	demonstrate that is possible to establish clinically relevant age dysmaturity scores
Scher et al., 2003	59 born ≤ 32 GA 50 fullterm	28-70,6	Sleep	24-channel EEG	arousal,REM, QS,sleep cycle length,β, spectr correllx/rx respiratory ratio	all 7 measures results required to best discriminate between cohorts
Iovleva et al., 2004	11 born 38-41 GA 12 born 34-36 GA 9 born 30-33 GA	19-30 days				Major power in 1 group of δ, θ, α. Higher coherence in δ range; in immature infants higher coherence in α and θ
de la Cruez et al., 2007	21 preterm and fullterm	32- 37 37- 42 42 - 47	QS, AS	Fp1, Fp2, C3, C4, T3, T4, O1, O2, average ref	coherence analysis, linear (AVL) and non-linear(AVN) average	β AVL greater in AS than in QS in preterm but the reverse in older full-term

4.2.2 Conclusive considerations

The meaning of the reported changes and differences in EEG spectral power are not well understood. Typically, investigators follow the short-term development of premature infants (1-2 years of age) in order to define the "normative" values of the obtained neonatal results as confirmed by the later normal neurocognitive development of these children. Furthermore, the highlighted differences with full-term infants were not beyond explored.

Adequate development used to has used to guarantee the validity as normative values of data recorded in neonatal period. To our knowledge, only one old study addressed a different question: Richards and coll. (1986) investigated the correlation between specific spectral parameters in neonatal periods and later sequelae, finding an association between lower amount of higher frequencies and worse outcome. Beside the need to establish normative values of spectral power data in early phase of life, there is of great importance the early detection of parameters predictive of later sequelae: the recognition of early indicators of risk factors in neonatal EEG. This is possible only by performing long lasting follow-up and by correlating neonatal features with later developmental outcome.

5. EVOKED POTENTIALS

Evoked potentials are brain electrical potentials changes recorded following presentation? of a stimulus. They are recorded from electrodes located on the surface of the scalp and become recognizable from the background activity thanks to the averaging technique, a summation of a sufficient number of stimulus-locked responses. The resulting waveform can be viewed as a sequence of waves; an oversimplified analysis of these waves is based on measurement of latency (the time of occurrence following the stimulus) reflecting mainly myelin function, and measurement of amplitude, which expresses the energy of the signal and is related to the number of activated neurons.

Evoked potentials comprise:

1) sensory evoked potentials, early electrical potential changes (within < 50 ms), evoked in response to sensorial stimulation and reflecting the activity of the sensory pathways ascending to cerebral cortex and the early obligatory cortical responses to specific sensory stimuli;

2) event-related potentials, late electrical potential changes, evoked in response to sensorial stimulation and internal processes and reflecting the activity of cortical associative areas. These evoked potentials are less reliable and more variable depending on subjects.

Most of the studies in the literature used an auditory stimulation (due to the better feasibility of this kind of stimulation in premature infants); thus auditory evoked potentials were treated in a dedicated paragraph.

5.1 Sensory Evoked Potentials

Sensory evoked potentials are electrical potential changes of sensory receptors, neural pathways and brain generated in response to external stimuli (De Graaf-Peters and Hadders-Algra, 2006). They are usually elicited by visual, auditory or somatosensory stimulation and reflect the activity of the sensory pathways ascending to cerebral cortex and early obligatory cortical responses to specific sensory stimuli.

Sensory evoked potentials allow the evaluation of the functional integrity of the sensory systems, from the periphery to cerebral cortex. In premature infants sensory receptors, peripheral nerves, the retina and visual pathways as well as the cochlea and auditory pathways are immature. The study of maturational changes of sensory evoked potentials in infants born preterm may offer a window on the development of thalamo-cortical connections and their ingrowths from the subplate into the cortical plate, during the early phases of brain development. Anatomic studies in the early seventies have already pointed out that the gradual shift in the synaptic and dendritic formation from predominantly deep to superficial cortical structures was underlying the immaturity of evoked potentials in premature infants; by contrast, brain maturation was explaining the consequent change of cortical electrical dipole (Kostovic´ and Judasˇ, 2002). Thanks to the advancements in knowledge of brain maturation we now know that the ingrowths of these connections from the subplate into the cortical plate occur at the time when very premature babies are viable to life; during this time so crucial for brain development cortical responses start to emerge following visual, auditory and somatosensory stimulation. The very long depolarization responses with negative polarity shown by all three sensory modalities occur at the time when studies demonstrate the thalamo-cortical fibers waiting in the subplate, whereas when the thalamo-cortical fibers grow into the cortical plate the

responses become more well-defined peaks, with positive polarity. In the period of coexistence of both the subplate and cortical plate intermediate waveforms may be recorded. Thus, in the early stages cortical responses appear to depend on the activation of transient structures and their endogenously generated intermittent activity; sensory input will only gradually begin to take part in shaping the developing circuitry (Vanhatalo and Kaila, 2006).

Visual Evoked Potentials (VEP). VEP responses can be recorded from 28 post-conception weeks; the visual evoked responses up to 32 post-conception weeks are characterized by a prominent negative wave at about 300 ms inconstantly preceded by a small occipital positive wave with a latency of about 200 ms. Between 35 and 40 post-conception weeks the positive wave increases in amplitude and is preceded by a small negative wave. This sequence of morphological changes of VEP responses development is the most reliable indicator of occipital cortex maturation; these changes have long been recognized to parallel the anatomical maturation of the human visual cortex (Takashima et al., 1980).

Somatosensory Evoked Potentials (SEP). Maturation shapes the evolving topography, latency and morphology of short latency SEPs in the period of the ingrowths of the thalamo-cortical fibers in the cortical plate, of the process of gyration of the cortex and of short cortico-cortical circuitry maturation (Vanhatalo and Lauronen, 2006). Thereafter SEPs development is characterized by two other coexisting phenomena with opposite effects: causing a progressive increase in conduction velocities and synchronization of potentials, myelinogenesis decreases the latency, while body growth has the opposite effect. The interaction of these two phenomena will explain the complex pattern of SEPs development in childhood (Suppiej, 2007). The maturational patterns described for CAEPs and VEPs is not

found in short latency SEPs used in routine clinical practice, but analyses of these later components demonstrated a maturational pattern similar to CAEPs and VEPs.

5.2 Auditory Evoked Potentials

5.2.1 Cortical Auditory Evoked Potentials (CAEP)

CAEP are electrical potential changes reflecting sequential activation of structures of auditory system from the cochlea to the primary and secondary projection areas in the auditory cortex. Measurements of CAEPs can be made prenatally but the uterine environment makes these measurements difficult. CAEP is one of the ontogenically earliest response and can be recorded as early as 24 weeks gestation in infants born prematurely; it is present at a time when cortical development is still very immature and afferent fibers are only beginning to be seen in the cortical plate (Krmpotic´-Nemanic´, 1983).

CAEPs recorded before the 36th gestational week are predominantly negative across the scalp and of opposite polarity to the response recorded in term infants (Wunderlich et al., 2006; Rotteveel et al., 1987; Weitzman and Graziani, 1968), as confirmed by neuromagnetic recordings (Lengle et al., 2001). As the premature infant approaches term the morphology of the response changes so that it is often of different polarities at the vertex compared to lateral sites (Weitzman and Graziani, 1968). Kurtzberg and colleagues (1984) described five maturational states of CAEP starting from preterm to term stage, characterized by negative responses in medial and lateral scalp sites in less mature infants (level I) and positive responses in medial and lateral scalp sites (level V) in more mature infants. This pattern of maturation is thought to be the result of earlier maturation of primary auditory areas followed by maturation of secondary areas, suggesting different developmental courses of the underlying generators.

At term, a large positive deflection at midline electrodes followed by a negativity has classically been reported?, the so called "P2- N2 complex" in response to a sound stimulation (Graziani et al., 1974; Rotteveel et al., 1987; Kushnerenko et al., 2002; Wunderlich et al., 2006). In most studies P2 and N2 peaks have been identified, even if the labelling of the components may vary (Kushnerenko et al., 2002). Earlier peaks, P1 and N1 may sometimes be seen but are much less frequently evoked (Weitzman e Graziani, 68; Kushnerenko et al., 2002; Fellman et al., 2004; Bisiacchi et al., 2009), due to the lower amplitude and to the greater variability (Kushnerenko et al., 2001).

CAEP of neonates has no resemblance to adult waveform and the infantile peaks do not correspond necessarily to adult peaks with the same names (Picton and Taylor, 2007). CAEP responses change again over the first years of life (Wunderlich et al., 2006), developing slowly during the childhood and adolescence into the adult multiphasic P1-N1-P2-N2 response. The prolonged age-related changes may then reflect maturational refinements of thalamic-cortical or cortico-cortical loops necessary for adult like auditory processing skills (Ponton et al., 2000).

It has been claimed that the early positive–negative response to repetitive standard tones may be the infant precursors of the P1–N1 adult components (Kushnerenko et al., 2002; Wunderlich et al., 2006), but this hypothesis remains speculative.

Intracortical recordings in adults suggest that the cortical generator of P1 is located in the lateral portion of Heschl's gyrus, i.e. secondary auditory cortex (LieÈgeois-Chauvel et al., 1994). N1 component is known to be the sum of at least three sub-components (Näätänen and Picton, 1987): (1) a fronto-central negativity originating bilaterally in the superior temporal cortex, typically labeled N1b. Orderly tonotopic shifts in the site of generation as a function of stimulus frequency indicates that the N1b peak reflects activity originating from primary auditory cortex; (2) a widespread

centro-posterior negativity reflecting modality non-specific activity, and (3) the T-complex at temporal sites.

Studies in non-human mammals have shown that primary auditory cortex is the cortical termination of the lemniscal pathway originating in the central nucleus of the inferior colliculus (Ponton et al., 2000). This pathway is activated exclusively by auditory stimuli and is tonotopically organized containing neurons with narrow frequency tuning curves and short latencies (Weinberger and Diamond, 1987). By contrast, the secondary auditory cortex is the major termination site for the ascending lemniscal-adjunct pathway which originates from the external nucleus of the inferior colliculus and terminate in cortical layer IV, but also produces activation of the medial nucleus of the medial geniculate body, which, in turn, projects diffusely to layer I of all auditory cortical areas. The lemniscal-adjunct system is not exclusively auditory; its neurons show broad frequency tuning, are non-tonotopically organized and have longer latencies than those in the lemniscal pathway (Weinberger and Diamond, 1987).

CAEP recorded in premature infants are thought to represent the activation of auditory cortical neurons mainly concerned with sensory sound perception, consistent with study identifying a location in the auditory cortex (Wunderlich et al., 2006; Lengle et al., 2001). However, the underlying generators of these auditory components, especially in neonates and premature infants, are actually object of debate. It has even been suggested that the CAEP recorded at this time represents cortical activity due to activation of non-lemniscal pathways arising from the reticular formation; at this time myelination of cochlear nerve and auditory brainstem structures is immature, the synchrony of neural response needed to obtain a reliable auditory brainstem potential cannot thus be seen until 28–29 post-conception weeks (Suppiej, 2007). The change from a predominant negative polarity to a predominant

positive polarity may reflect development of specific laminae of the cerebral cortex. Initially, the thalamo-cortical innervations is limited to lamina IV, and this generates a surface negative potential, whereas the following innervations of lamina III results in a shift to a surface positive potential (Novak et al., 1989).

5.2.2 Event-related potential (ERPs)

Unlike sensory evoked potentials, ERPs are long-latency potentials elicited in response to stimuli requiring some type of cognitive operation, thus providing a unique opportunity to evaluate higher-order cortical processes (Stapells and Kurtzberg, 1991; Fellman et al., 2004). Whereas evoked potentials reflect the activation of well- defined sensory pathways, ERPs are generated in parietal, temporal and frontal association areas, rending the identification of their neural generators very difficult. This make ERPs more variable and less reliable for clinical use in neonatal neurology. Research, however, showed that specific neuropsychological paradigms can elicit different ERP components, that, although variable, show characteristics according to the supposed underlying cognitive operation stimulated by the paradigm. Interesting examples of ERP components elicited within a specific paradigm are: - the MMN, an automatic change detection response; - the N170, a negative wave elicited in response to facial stimuli; - the P300 wave, a late positive wave recognizable when a subject discriminates a stimulus target from series of standards, etc…(Picton and Taylor, 2007).

Like sensory evoked potentials, ERPs can be elicited using any kind of sensory modality but auditory stimulation is most frequently used with neonates, because it can be recorded even in sleep (Sheldon, 1996). Research in the last decade mainly focused on several versions of the auditory change-detection paradigm, the so called "odd-ball". The typical experimental procedure consists of occasionally replacing

repetitive "standard" stimuli by a physically "deviant" stimulus with tones and speech sounds as the more frequently used stimuli (Picton and Taylor, 2007). Responses elicited by the standard stimuli allow evaluation of sound perception, but meanwhile a memory trace is automatically formed holding a representation of the standard stimulus (Fellman and Huotilainen, 2006); when the deviant occurs, it is automatically compared to the memory trace and if a difference is detected the mismatch negativity (MMN), a small negative wave, is elicited (Na¨a¨ta¨nen, 1978, 1990, 1992; Alho et al., 1990; Cheour et al., 2002a; Fellman and Huotilainen, 2006). Thus, the MMN is an automatic and pre-attentive cortical response that reflects auditory discrimination, sensory memory and automatic attention (Na¨a¨ta¨nen, 1990, 1992).

In adults the generators are thought to be in auditory cortices, specifically in the supratemporal plane, and in the right frontal area (Alho, 1995).

MMN has been said to develop rather early in comparison to other event-related potential waves (Leppa¨nen et al., 2004). It has even been suggested to be the ontogenetically earliest discriminative response of the human brain (Cheour-Luhtanen et al., 1995). Indeed, an adult-like MMN response has been reported in preterm and full-term newborns and in early infancy (Na¨a¨ta¨nen et al., 1978; Alho et al., 1990; Kushnerenko et al., 2001; Fellman V, Huotilainen, 2006; Novitski et al., 2007). However, MMN in newborn infants remains controversial. Although the original studies showed a frontal-negative difference wave, its scalp topography differed from adults (Alho et al., 1990); other studies have recorded a positive difference wave (Dehaene-Lambertz and Gliga, 2004); finally, some authors failed to find a MMN response in normal term infants (Alho et al., 1990; Kurtzberg et al., 1995). Possible explanations of the discrepant findings in the literature are probably differences in methodology and interindividual variability (Morr et al., 2002; Friederici et al., 2002; Suppiej et al., 2010). By comparing the change-detection ability in premature infants

reaching term or near term age with that in full-term, atypical pattern of maturation has been described in premature infants. Discriminative responses to deviant stimuli are more spread and delayed (Alho et al., 1990), smaller and more negative (Therien et al., 2004), atypical or even absent (Fellman et al., 2004), or present only in active sleep (Suppiej et al., 2010). Notably, the immature morphology persists even when the age at measurement is corrected to term (Fellman and Huotilainen, 2006). Leppa¨nen et al. (1997) reported ERPs maturational changes in the MMN paradigms consisting in positive responses in more mature infants and concluded that maturational patterns could affect ERPs measured in the MMN paradigm; these effects could depend on changes in the infant brain during the pre- and perinatal stages of development (Leppa¨nen et al., 2004), but the developmental timetable of the auditory cortex and the main neural generators of the neonatal MMN remain at present largely unknown (Trainor et al., 2003).

We can hypothesize that the observed changes in amplitude and polarity of MMN could reflect the rapid development of thalamo-cortical connections, cortical lamination, and synaptic activity in early development (Trainor et al., 2003).

5.2.3 Clinical applications

As above described (see Chapter 1), premature infants are at high-risk for cognitive outcome. Evoked potentials are proposed to be early indicators of risk factors. Bisiacchi and colleagues (2009) found a positive– negative complex in response to a pure tone of 1000 Hz. Although infants were evaluated at the same post-conception age, those born at an earlier age (<30 gestational weeks) demonstrated smaller cortical responses (a reduced N2 mean amplitude) than older ones (>30 gestational weeks). This could be due to a more immature primary cortical organization in children born under 30 weeks of gestational age.

In children deficits in CAEP and auditory ERPs have been found in association with learning disabilities, autism, Asperger syndrome, cleft palate, depression and attention deficit disorder (Leppa¨nen et al., 1997; Kujala and Na¨a¨ta¨nen, 2001; Lepisto et al., 2006). Correlation was found between ERPs and the developmental index at 2 years of age (Fellman et al., 2004) and between abnormal ERPs in the neonatal period and later deficiencies in language processing skills (Molfese 2000). Furthermore, Mikkola and coll. (2007) found a diminished P1 amplitude in premature children at five years of age, suggesting an altered primary auditory processing. Therefore, the hypothesis was suggested that abnormal ERP at birth and during the first years of life may indicate later cognitive dysfunction (Fellman et al., 2004).

In this view, the evoked potentials techniques may reflect a direct measure of cortical functioning, thus providing a unique window on the brain at work during the early phases of development, in normal and pathological conditions being, at present, regarded as possible markers of brain damage during the early phases of development (Tommiska et al., 2003).

6. AIMS OF THE STUDY

This work aims to investigate early differences in electrophysiological brain activity of premature infants experiencing different developmental pathways, which may be suggestive of risk factors for later sequelae.

More specifically, we conducted cross-sectional and longitudinal investigationgs at early crucial phases of development (35 and 40 weeks post-conception - PCA) in order to identify differences in cerebral activity between premature infants born at different gestational ages (GA) and full-term infants, using EEG at rest and CAEPs. We further aimed to correlate the neonatal data with later neurodevelopment.

The research is divided into three studies.

Study 1. EEG spectral activity was recorded at 35 PCA in premature infants and compared between groups of infants born at different GA. The results were correlated with developmental scores obtained at 12 months corrected age.

Study 2. A subgroup of infants of Study 1 repeated the EEG recording at 40 PCA. EEG spectral activity of this subgroub was compared longitudinally within groups and further with those of a group of full-term infants.

Study 3. CAEP was recorded in active sleep in response to trains of stimuli (1000 Hz paradigm 1, 500 Hz paradigm 2) at 35 PCA in premature infants and compared between groups of infants born at different GA. The results were correlated with developmental scores obtained at 12 months corrected age.

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

Behavioral state

Sleep states (active, quiet and indeterminate sleep) in premature infants can be detected by behavioral observation of the infant as well as with the electroencephalographic staging (Curzi-Dascalova et al., 1988; Mercuri et al., 1995). In infants older than 36 weeks the behavioral state was classified according to the criteria proposed by Anders et al. (1971) and adopted by Shepherd et al. (2000) for scoring sleep states while those below 36 weeks were classified by the behavioral criteria of Mercuri et al. (1995).

7. STUDY 1

7.1 Aim

To investigate early differences in spectral EEG activity between premature infants born at different GA as they attained 35 PCA and the association of the results with developmental scores obtained at 12 months corrected age.

7.2 Methods

7.2.1 Population

Twenty-seven premature infants (mean 30 GA, range 23–33) admitted to the neonatal intensive care unit of the Department of Paediatrics of the University of Padua were recruited before discharge. Infants with cranio-facial malformations, genetic syndromes, clinical evidence of neonatal encephalopathy, ultrasound evidence of intra-ventricular hemorrhage or periventricular cystic leukomalacia, as well as those treated with drugs with central nervous system effect were not included. All recordings took place between 10.30-12 h am, a time slot in which no medical or feeding procedure is carried out. PCA was computed as the sum of GA at birth and the period of extra-uterine life elapsed from birth to EEG recording.

Thirteen premature infants (mean 35 GA, range 34 –35) admitted to the well-baby nursery of the Department of Paediatrics of the University of Padua were recruited at 3th -5th day of life.

All forty premature infants were eligible and underwent EEG recording at 35 PCA (range 34- 36). Infants were grouped according GA as follows: 11 "extremely low gestational age" (ELGA, 23–27^{+6}), 16 "very low gestational age" (VLGA, 28–31^{+6}) and 13 "low gestational age" (LGA, 34-35) infants.

7.2.2 Methodology

Data acquisition. Five bipolar channels according to the 10–20 International System were recorded: Fp1-Fp2, C3-C4, C3-T3, C4-T4, O1-O2; Fpz as ground electrode. All electrode impedances were less than 10 KΩ and balanced. Artifacts related to movement of the infant or replacement of an electrode were removed when impedance value was >10 kΩ.

Data analysis. Data were analyzed with an EBNeuro dedicated software (EBNeuro/Florence, Italy). Data were filtered using a 0.5 Hz high-pass filter and a 30 Hz low-pass filter. After multiplication with a rectangular window function the time series was transformed into the frequency domain using a Fast Fourier Transform algorithm. The frequency spectrum was determined for each 2-second segment (50% overlapping) and averaged over the complete 40 minutes recording (range 35-45 minutes). Recordings were carried out at rest and each comprised a segment of active, quiet and indeterminate sleep.

Frequency spectrum was divided into the following bands: δ (0.5-4 Hz, comprising δ1 0.5-1 Hz and δ2 1-4 Hz), θ (4-8 Hz), α (8-13 Hz) and β (13-20 Hz). From the transformed signal the absolute power (defined as the integral of all powers within the frequency band, expressed in μV^2) was calculated. Because absolute spectral power between subjects may vary considerably, spectral values were standardized for total power and expressed as relative power (defined as the ratio of absolute band power to total power of all bands, expressed in percentage) and for each mean bands power and expressed as z scores (defined as the difference between absolute band power and mean power of each band, divided by standard deviations of band).

7.2.3 Behavioral developmental index at 12 months corrected age

The rate of development was followed during infancy and early childhood. As infants attained 12 months, corrected age underwent a standardized developmental scale, The Griffiths Mental Development Scales, GMDS – Revised. The scale returned a general index, which measures the rate of development, and five specific sub-scales scores relative to locomotor, relational/personal self autonomies, language, manual ability and performance development. The scores were expressed in quotients (mean=100, standard deviation=15).

Twenty infants reached 12 months corrected age and underwent the Griffiths Mental Development Scales.

7.2.4 Statistical analysis

Given the predominant role of medial areas emerged in studies on early development (Kurtzberg et al., 1984; Suppiej et al., 2010), only central and centro-temporal sites (C3-C4, C3-T3, C4-T4) were analyzed. Clinical data (male rate, ph, Apgar Scores and weight) were compared between groups in order to exclude possible confounding effects. Differences in absolute and standardized frequency power values between groups were evaluated using a MANOVA. Results were further specified by Bonferroni's post hoc tests. Where the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. In order to avoid mistakes due to splitting infants into groups, spectral power values were correlated with GA using a Pearson's r correlation; thus, in order to investigate the self-organizing characteristic of spectral power values, a cluster analysis was performed on standardized spectral values. Standardized spectral power values and GA were correlated with Developmental scores at 12 months corrected age with a Pearson's r correlation.

Data were statistically analyzed with SPSS 13.1 (Inc, Chicago, IL, USA). A p-value < 0.05 was considered significant.

7.3 Results

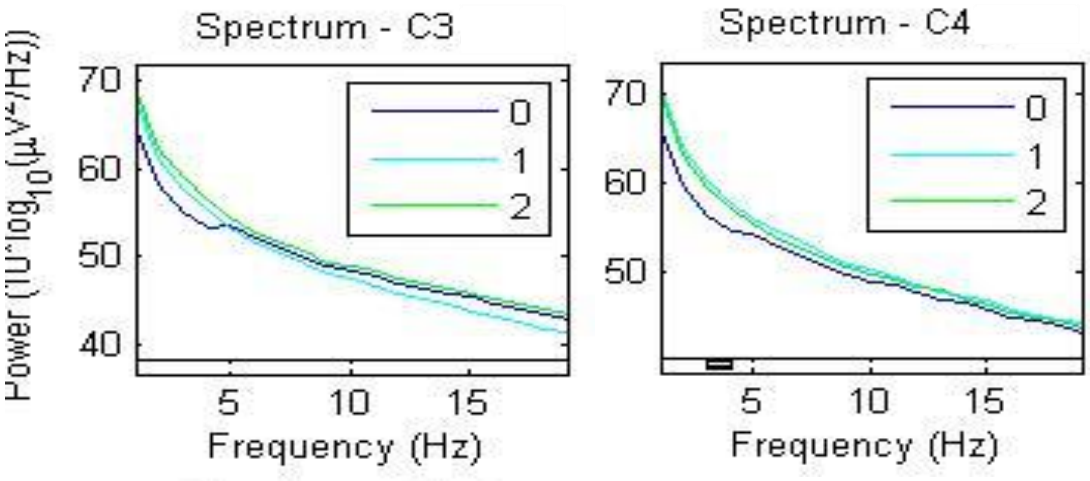
7.3.1 Neonatal data

Male rate, ph and Apgar Scores did not differ between groups. As expected, weight at birth correlated with GA (r=.83, p=.001). All EEG recordings of premature infants showed the background patterns generally found at this age: *tracé continue* (continuous EEG activity of similar voltage and frequency) and *tracé discontinue* and *tracé alternant* (alternating segments of activity and quiescence). None of the EEG recordings showed evidence of neonatal seizures.

The main frequency recorded (over 90% of the total spectral power recorded) fell in δ frequency range.

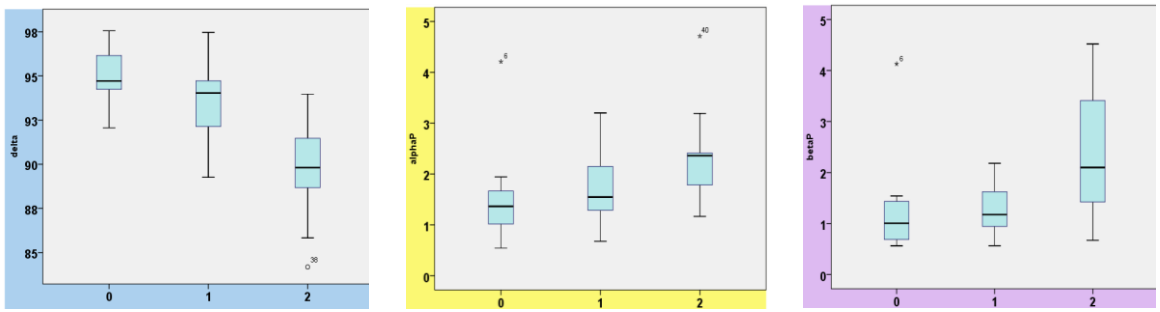
Absolute power. No significant differences between groups were found in absolute power on C3-C4, C3-T3, C4-T4. Values on C3 and C4 sites in the three groups are shown in Figure 7.1.

Figure 7.1. Absolute spectral power values in the groups: ELGA (0), VLGA (1), LGA (2) on C3 and C4 sites



Relative power. Differences between groups were found in δ (p=.03), α (p=.04) and β (p=.01) frequency ranges on C3-C4 site (Figure 7.2). Bonferroni's post hoc comparisons revealed a statistical difference only between ELGA and LGA groups in δ (p=.03), α (p=.04) and β (p=.01) frequency ranges. The VLGA group did not differ from other groups. No differences were found in θ frequency range.

Figure 7.2. Relative spectral power values expressed in percentage (%) for δ (a), α (b) and β (c) frequency range in the groups: ELGA (0), VLGA (1), LGA (2)



a) b) c)

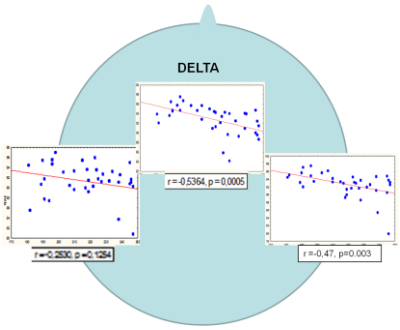
Legend: 0: extremely low gestational age (ELGA <28 GA); 1: very low gestational age (VLGA 28 – 31^{+6} GA); 2: low gestational age (LGA 34 – 35 GA). Frequency bands: δ (0,50-4 Hz), α (8-13 Hz), β (13-20 Hz).

No significant differences between groups were found on C3-T3 and C4-T4 sites.

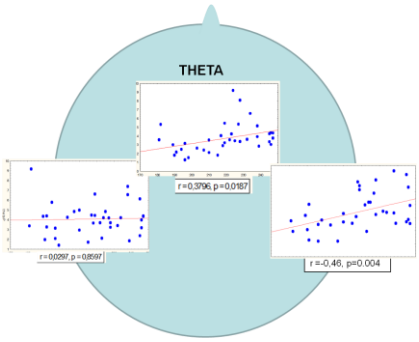
Linear correlations. Correlation was found between relative spectral values and GA for δ (C3-C4: r=-.54, p=.0005; C4-T4: r=-.47, p=.003), θ (C3-C4: r=.37, p=.01; C4-T4: r=.56, p=.004), α (C3-C4: r=.44, p=.005), β (C3-C4: r=.56, p=.0003; C3-T3: r=.35, p=.03; C4-T4: r=.33, p=.04) frequency ranges. R, p values and scatter plots are reported in Figure 7.3.

Figure 7.3. Linear correlation between GA and frequency ranges (δ, θ, α, β) in C3-C4, C3-T3, C4-T4: a) r and p values, b) scatter plots

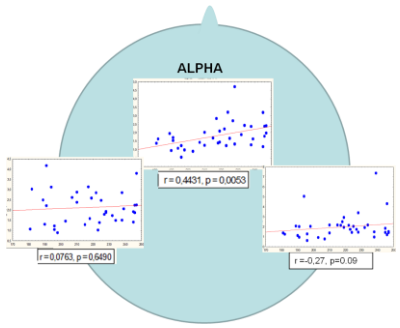
DELTA		
CHANNEL	GESTATIONAL AGE	
	r	p value
C3-C4	**-,53**	**.001**
C3-T3	-,25	.12
C4-T4	**-,47**	**.003**



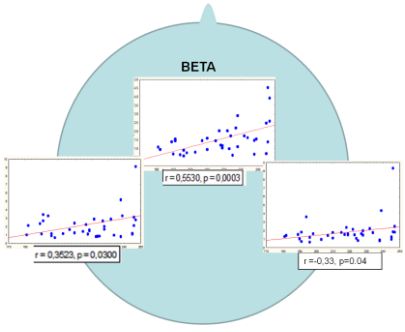
THETA		
CHANNEL	GESTATIONAL AGE	
	r	p value
C3-C4	**,37**	**.019**
C3-T3	,02	.86
C4-T4	**,46**	**.004**



ALPHA		
CHANNEL	GESTATIONAL AGE	
	r	p value
C3-C4	**,44**	**.005**
C3-T3	,07	.64
C4-T4	,27	.09



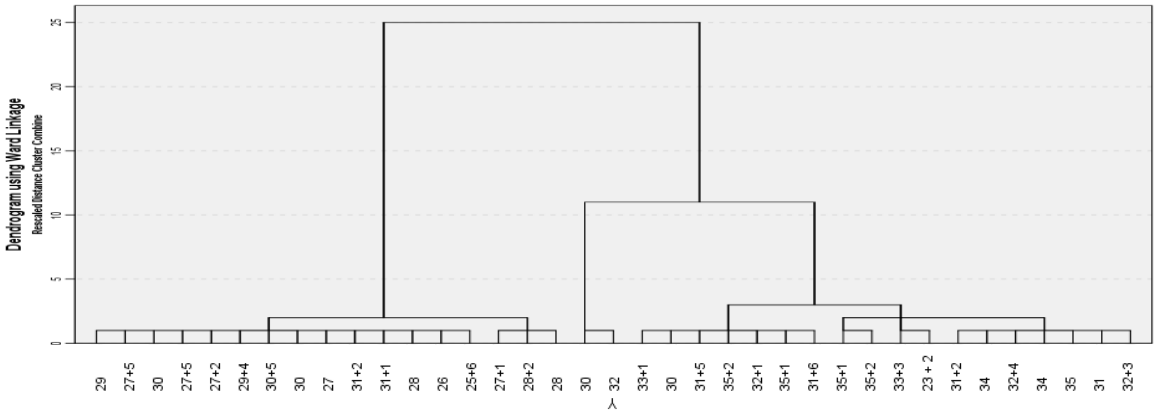
BETA		
CHANNEL	GESTATIONAL AGE	
	r	p value
C3-C4	**,55**	**.0001**
C3-T3	**,35**	**.03**
C4-T4	**,33**	**.04**



a) b)

Cluster analysis. The resulting dendrogram (Figure 7.4) showed the emergence of the natural grouping of the relative power spectral values into two main clusters of distinct subjects according to GA at birth on central site (C3-C4).

Figure 7.4. Dendrogram resulting from cluster analysis performed on δ, θ, α and β relative power spectral values on C3-C4.



7.3.2 Behavioral developmental index at 12 months corrected age

Twenty premature infants (seven ELGA, seven VLGA, six LGA) attained 12 months corrected age. Group's mean Griffith's scores resulted adequate for age; only one child fell in the borderline range of normality. Individual performance on Griffith's subscales scores are shown in Figure 7.5 and differentiated for groups in Figure 7.6.

Figure 7.5. Griffith's subscales scores of the 20 patients on A (gross-motor), B (relational/ personal self autonomies), C (language), D (manual ability) and E (performance).

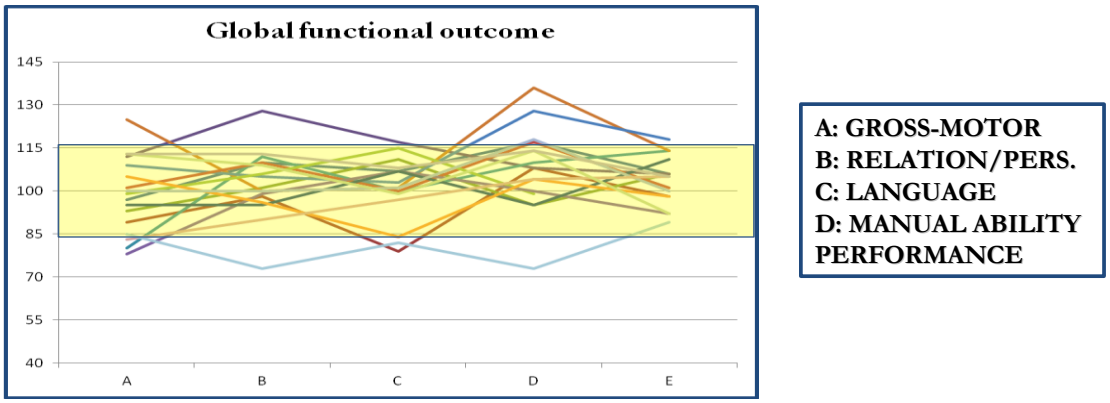
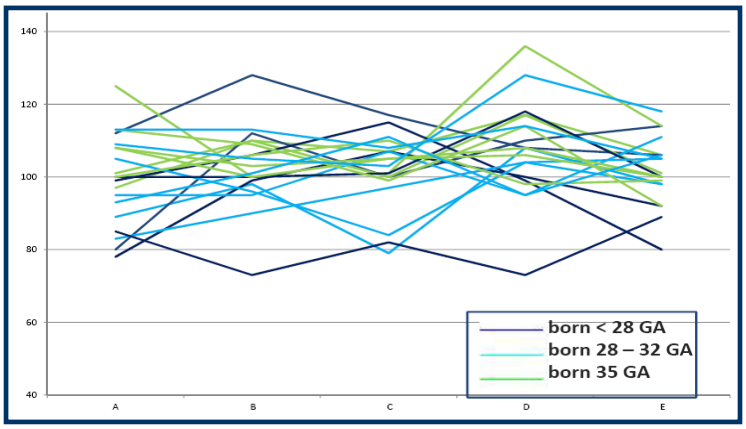


Figure 7.6. Griffith's subscales scores in the three groups of the 20 patients (ELGA born < 28 GA, VLGA born 28-32 GA, LGA born 34-35 GA) on A (gross-motor), B (relational/ personal self autonomies), C (language), D (manual ability) and E (performance).



The correlations between Griffith's subscale and total scores and GA are presented in Table 7.1. Outcome correlated with GA: higher GA was were associated with better performance in motor area, both gross-motor (r=.67, p=.009) and manual ability (r=.67, p=.009).

Table 7.1. R' Pearson and p-value of correlations between Griffith's subscale and total scores and GA

Outcome Scores	r Pearson	P value
A – Gross-motor	**.67**	**.009**
B – Relation/Personal		*ns*
C – Language		*ns*
D – Manual ability	**.67**	**.009**
E – Performance		*ns*
Total		*ns*

The correlations between Griffith's subscale and total scores and relative spectral values on C3-C4 site are presented in Table 7.2. Relational and personal self

autonomy skills sub-scale score correlated negatively with δ and positively with β and α relative power values.

Table 9.2. R' Pearson and p-values of correlation between Griffiths subscale and total scores and relative power frequency bands on C3-C4 site.

	δ		θ		α		β	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
A – Gross-motor		*ns*		*ns*		*ns*		*ns*
B –Relat/Person.	**-.60**	**.01**		*ns*	**.63**	**.006**	**.63**	**.007**
C – Language		*ns*		*ns*		*ns*		*ns*
D – Manual abil.		*ns*		*ns*		*ns*		*ns*
E – Performance		*ns*		*ns*		*ns*		*ns*
Total		*ns*		*ns*		*ns*		*ns*

No correlation between Griffith's subscale and total scores and spectral power values recorded on C3-T3 and C4-T4 sites were found.

7.4 Discussion

Premature infants experiencing different developmental pathways exhibited differences in spectral power values at 35 post-conception age, the differences of whichappear strongly associated with short-term development of relational and personal self-autonomies competencies.

Infants born at lower ages showed a higher amount of δ power values and a lower amount of higher frequencies (α and β). The result was not a simple chance finding in neonatal period, but seemed to be associated with later development: relational/personal self autonomies competencies at 12 months inversely correlated with the amount of δ power and positively with the amount of α and β power values.

Interestingly, all results were found only on central sites (C3-C4), confirming previous reports of earlier cortical development of the midline areas (Kurtzberg et al., 1984; Suppiej et al., 2011).

Studies about visual inspection of EEG showed that electrical activity changes progressively in the third trimester of gestation, with specific pattern of activity linked to specific neuro-functional changes, and age-specific patterns (Vecchierini et al., 2007; André et al., 2010). Total spectral power decreases with age, with a shift from the lower high-amplitude waves to the higher frequency low-amplitude content of the EEG (Vecchierini et al., 2007; André et al., 2010). Data on cerebral electrical activity development of "healthy" premature infants showed that absolute and relative δ spectral power measures decrease at increasing post-conception age, with an increase of relative α and β spectral power, which however becomes a noteworthy change only after the 35 post-conception weeks (Scher et al., 2005).

The higher amount of relative δ power found in our study in infants born at lower gestational ages may reflect a delayed relative reduction of the power or a transient increase with protective value. It may be the result of longer exposition to overstimulation or of subclinical pathological conditions. Slow activity is thought to play a major role in the functional and structural shaping of neuronal circuitries in immature brain tissue (Penn and Shatz,1999; Garaschuk, et al., 2001) and the persistence of slow delta waves after 33-34 weeks of gestation is related to adverse outcomes (Hayakawa et al., 1997).

No differences were found in the θ frequency range beteen groups; however, a positive correlation was found with gestational age, indicating a increase of power in this frequency range in those infants with higher gestational age at birth. High-voltage rhythmic temporal θ bursts appear frequently between 27-28 and 32 weeks post-conception weeks and diminish progressively later (Selton et al., 2000; Vecchierini et

al., 2003; André et al., 2010). These high-amplitude θ rhythms are characteristic of the temporal localization for very premature infants and are considered physiological (Hughes et al., 1997); they reflect on spectral power as a large amount of θ spectral power. The finding of a lower amount of θ spectral power in infants born at lower gestational ages in the period at which a preponderance of θ is physiological is suggestive of an interference of the normal processes undergoing these high-voltage rhythmic temporal θ bursts.

Differences were found also in less represented frequencies, β and α. The maturation of intracortical interneuronal circuits and of inhibitory neurotransmission is required for the generation of a robust higher frequency activity, believed to be essential for high cognitive functions (Vanhatalo and Kaila, 2006).

B frequency is classically considered related to cognitive processes and it appears as a main frequency only after the formation of short cortico-cortical circuitry (after 35 gestational age). At the time of our recording, β frequency is still very underrepresented compared relative to others frequencies. However, differences occur between groups, indicating a possible delayed development of intra-cerebral circuitry in ELGA compared to more mature infants at birth; these results may reflect even adaptive strategies to extrauterine life (Scher et al., 1995).

The amount of α frequency power differed between ELGA and LGA groups. In the immature brain frequencies in the range of α do not resemble morphology and localization of α recorded in the adult and their functions are not well understood, but their importance as marker of development was demonstrated (Scher, 1997).

Given the rapidity of changes in the third trimester of gestation, markers of neurophysiological variations in the neonatal period have been difficult to establish, except for those infants with serious neurological problems. Preterm birth has been used as a prototype of a general marker for biological deviance that could affect

behavioral outcome (Beckwith and Parmelee, 1986). It is, however, a very general marker including a wide range of risk factors from severe handicap to well doing.

Our results showed that even at an early stage subtle differences in maturation of electrical activity are recognizable in premature infants using spectral analysis of EEG. The differences in electrical brain activity reflected by spectral analysis do not have a well-defined meaning, but they may be interpreted as a different cortical organization in infants experiencing different pathways of development in a crucial phase. These alterations are thought to occur early and seem to involve especially extremely low gestational age infants, pointing to the importance of paying special attention to the more vulnerable brain of those infants born at lower gestational age.

Unlikely later cognitive functions, motor and relational/personal self autonomies skills are main acquisitions in the first year of life. Both gestational age and spectral power values seem to be associated respectively with the development of motor functions and of relational/personal self autonomies.

Data on minor impairments in premature infants indicate a great incidence of developmental coordination and psychiatric disorders (Bhutta et al., 2002; Aylward, 2002; Johnson, 2007). Neonatal and follow-up results are coherent and when considered in this theoretical reference picture, the strong association between Griffith's scores and spectral power values suggest the possibility to early individuate indicators of risk factor for later sequelae.

Although the impact of early alteration in spectral power values on short-term development is yet evident, the implications for later development are unclear. Long-term follow-up is going to highlight the real significance of these results.

In conclusion, we performed spectral analysis EEG recordings in premature infants born at different gestational ages at the time at which they attained the same 35 post-conception age. We did not find any differences in total spectral power,

indicating the relative power as a more sensitive tool in detection of subtle differences in a heterogeneous group of premature infants at a same post-conception age. Spectral power analysis of the EEG, easily performed bedside, provides information about brain function and help to identify possible factors which interfere with normal brain development. The present work suggests that maturation in early stages may be assessed by main changes in the δ (0.5-4 Hz) spectral frequency band of the EEG, but also by less represented frequencies, such as β (13-30 Hz) and α (8-13 Hz); furthermore, these changes seem associated with short-term development.

8. STUDY 2

8.1 Aim

Study 2 aimed to investigate the spectral EEG activity development between 35 and 40 post-conception age of a subgroup of infants of Study 1 longitudinally. The spectral activity at 40 post-conception age was further compared with those of a control group of healthy full-term neonates.

8.2 Methods

8.2.1 Population

A subgroup of infants (five ELGA and five VLGA) of Study 1 was recruited to Study 2 and was retested as they attained term age (40 PCA). Ten full-term neonates (mean GA 40, range 39–40) admitted to the well-baby nursery of the Department of Paediatrics of the University of Padua were recruited at 3th -5th day of life as control group.

8.2.2 Methodology

Study 2 used the same methodology of Study 1 (see 7.2.2 paragraph).

8.2.3 Statistical analysis

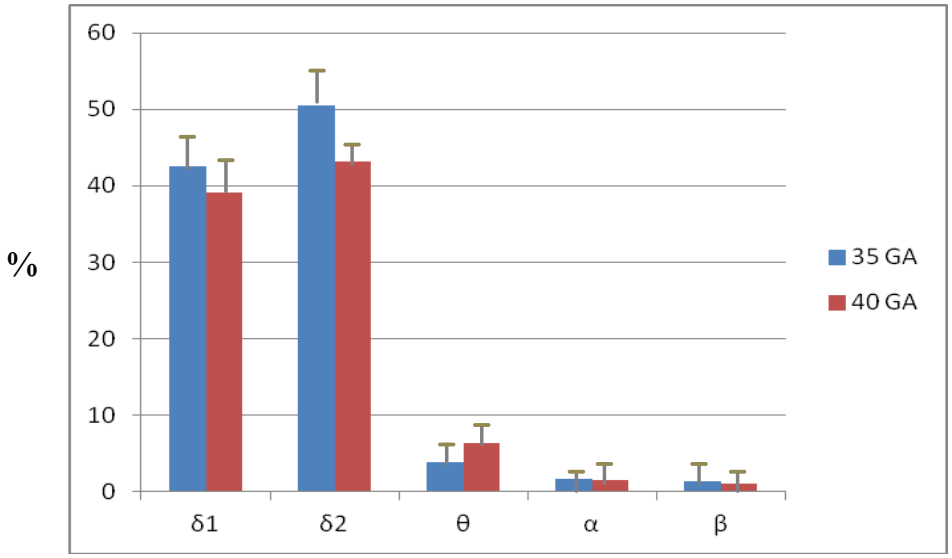
Given the predominant role of medial areas found in Study 1 and documented in studies on early development (Kurtzberg et al., 1984), only central sites (C3-C4) were analyzed. Differences in standardized frequency power values on time (35 and 40 GA) between groups were evaluated using a repeated measures ANOVA. Results were further specified by Bonferroni's post hoc tests. Where the assumption of sphericity was violated, the Greenhouse-Geisser correction was used.

Data were statistically analyzed with SPSS 13.1 (Inc, Chicago, IL, USA). A p-value < 0.05 was considered significant.

8.3 Results

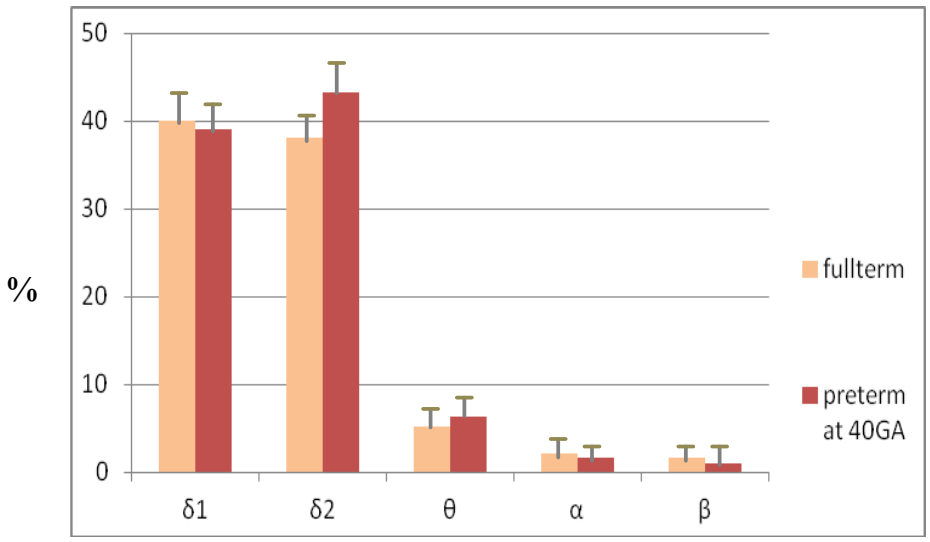
By comparing the relative spectral power values recorded at 35 and at 40PCA a significant effect was found only for δ frequency range (p=.03) (Figure 8.1).

Figure 8.1. Relative spectral power values (%) in δ1 (0,5-1 Hz), δ2 (1-4 Hz), θ (4-7 Hz), α (8-13 Hz), β (13-20 Hz) at 35 and 40 GA in premature infants.



By comparing the relative spectral values recorded in premature infants at 40 PCA with those of full-term neonates we did not find any significant differences (Figure 8.2).

Figure 8.2. Relative spectral power values (%) in δ1 (0,5-1 Hz), δ2 (1-4 Hz), θ (4-7 Hz), α (8-13 Hz), β (13-20 Hz) in premature infants at 40 GA compared to full-term neonates.



8.4 Discussion

By following a subgroup of premature infants from 35 to 40 post-conception age a significantly reduction in δ frequency range is shown, while changes in other frequency ranges did not reach statistical significance. Interestingly, by comparing these infants at 40 post-conception age with full-term neonates no statistically differences in spectral EEG activity emerged.

The decrease in δ amount is in line with previous studies about development of spectral EEG activity in premature infants at increasing age (Niermarkt et al., 2011; Scher, 1997; Okumura et al., 2003). Changes in low frequency range are the most stable and reliable in neonatal period, due to the higher prevalence of δ frequency in EEG spectra contents. More subtle changes (as differences with full-term neonates or changes in higher frequency ranges) are difficult to emerge in a small sample (10 premature *vs* 10 full-term infants).

Despite the small sample it is possible that earlier neurophysiological evaluations allow to photograph brain functionality during a critical window. Subtle alterations present in brain activity at any stage may not be more evident later: EEG disturbances are more likely to be documented if recordings are obtained more closely to the time when the disturbance to brain function occurs (Scher et al., 1995), while abnormal patterns become progressively less demonstrable during the convalescent period.

It would be interesting to investigate in a larger group of infants the development at this early phase, both longitudinally and cross-sectionally, in order to better explain the real significance of the results found in this study.

In conclusion, we followed a small subgroup of infants of Study 1 from 35 to 40 post-conception age. Interestingly, 35 post-conception age appears to be a more crucial phase in establishment of alterations in brain functionality.

9. STUDY 3

9.1 Aim

To investigate early differences in CAEP between premature infants born at different gestational ages as they attained 35 post-conception age and the association of the results with developmental scores obtained at 12 months corrected age.

9.2 Methods

9.2.1 Population

Twenty-four premature infants (mean 30 gestational age – GA, range 23–33) admitted to the neonatal intensive care unit of the Department of Paediatrics of the University of Padua were recruited before discharge. Infants with cranio-facial malformations, genetic syndromes, clinical evidence of neonatal encephalopathy, ultrasound evidence of intra-ventricular haemorrhage or periventricular cystic leukomalacia, abnormal evoked otoacoustic emissions, as well as those treated with drugs with central nervous system effect were not included. All recordings took place between 10.30-12 h am, a time slot in which no medical or feeding procedure are carried out. PCA was computed as the sum of GA at birth and the period of extra-uterine life elapsed from birth to neurophysiologic examination.

Twelve premature infants (mean 35 GA, range 34 –35) admitted to the well-baby nursery of the Department of Paediatrics of the University of Padua were recruited at 3th -5th day of life.

Infants underwent continuous EEG recording under auditory stimulation, in order to evoke CAEP, at 35 PCA (range 34-35 GA). Infants were grouped according GA as following: 10 "extremely low gestational age" (ELGA, 23–27+6), 14 "very low

gestational age" (VLGA, 28–31+6) and 12 "low gestational age" (LGA, 34-35) infants. Recording under auditory stimulation (8 minutes duration) started as soon as an active sleep state was identified.

9.2.2 Methodology

Stimuli and procedure. Two auditory stimulations were used; the first consisted of 1000 Hz tones and the second of 500 Hz tones, delivered binaurally via headphones. The tone duration was 100 ms (5 ms rise and 5 ms fall time), the intensity 75 dB nHL for 1000 Hz tones and 85 dB nHL for 500 Hz tones. The inter-stimulus interval (ISI) was randomly varied between 600 and 900 ms (offset-to-onset). The experimental design consisted of 300 tones for each infant for each paradigm.

Data acquisition. EEG was recorded at Fp1, Fp2, Fz, Cz, C3, C4, T3, T4, O1 and O2 and referenced to the bilateral linked ear lobes; Fpz as ground electrode. All electrode impedances were less than 10 KΩ and balanced. Artifacts related to movement of the infant or replacement of an electrode were removed when impedance value was >10 kΩ. Vertical eye movements were monitored with a bipolar electro-oculogram (EOG). Data were amplified and digitized with a sampling frequency of 512 Hz. The band-pass filtering was 0.1–100 Hz.

Data analysis. Data were analyzed with EEGlab 11 (Matlab toolbox for EEG processing). EEG and EOG were filtered offline (0.5 Hz high pass and 20 Hz low pass). Artifact rejection for the EEG and EOG was performed with 100 mV as the delta criterion. Epochs were 600 ms in duration, including a 50 ms pre-stimulus baseline interval, and divided for statistical analysis into time windows of 100 ms (50-150, 150-250, 250-350, 350-450 ms).

9.2.3 Behavioral developmental index at 12 months corrected age

The rate of development of the infants was followed during infancy and early childhood. As infants attained 12 months corrected age, they underwent a standardized developmental scale, The Griffiths Mental Development Scales, GMDS – Revised. The scale returned a general index measuring the rate of development and five specific sub-scales scores relative to locomotor, relational/personal self autonomies, language, manual ability and performance development. The scores were expressed in quotients (mean=100, standard deviation=15).

Twenty infants reached 12 months corrected age and underwent the Griffiths Mental Development Scales.

9.2.4 Statistical analysis

Given the predominant role of medial areas emerged in Study 1 and documented in studies on early development (Kurtzberg et al., 1984; Suppiej et al., 2010), only central sites (Cz, Fz) were analyzed. Clinical data (male rate, ph, Apgar Scores and weight) were compared between groups in order to exclude possible confounding effects. Differences in CAEPs on time windows (50-150, 150-250, 250-350, 350-450 ms) and on sites (Fz, Cz) between groups were evaluated using a repeated measures ANOVA. Results were further specified by Bonferroni's post hoc tests. Where the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. CAEP was correlated with Developmental scores at 12 months corrected age with a Pearson's r correlation.

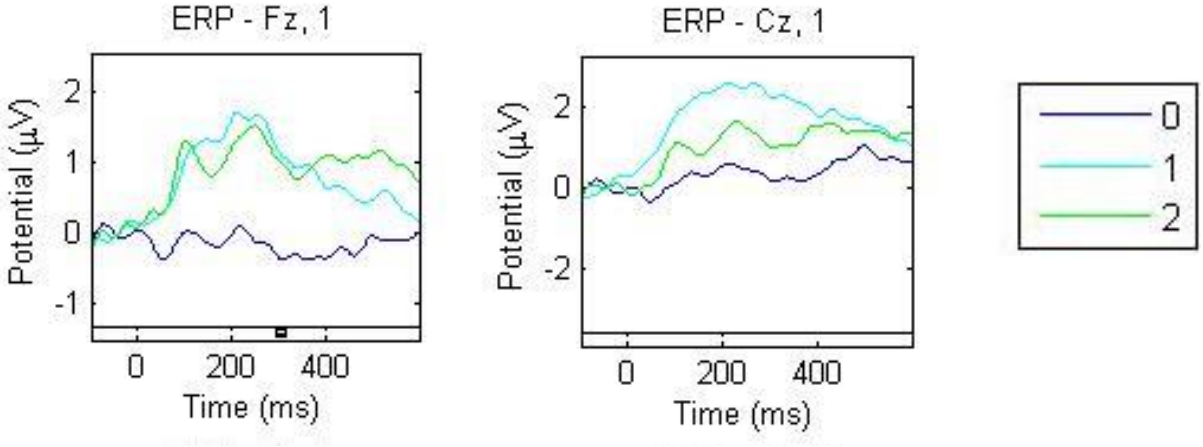
Data were statistically analyzed with SPSS 13.1 (Inc, Chicago, IL, USA). A p-value < 0.05 was considered significant.

9.3 Results

9.3.1 Neonatal data

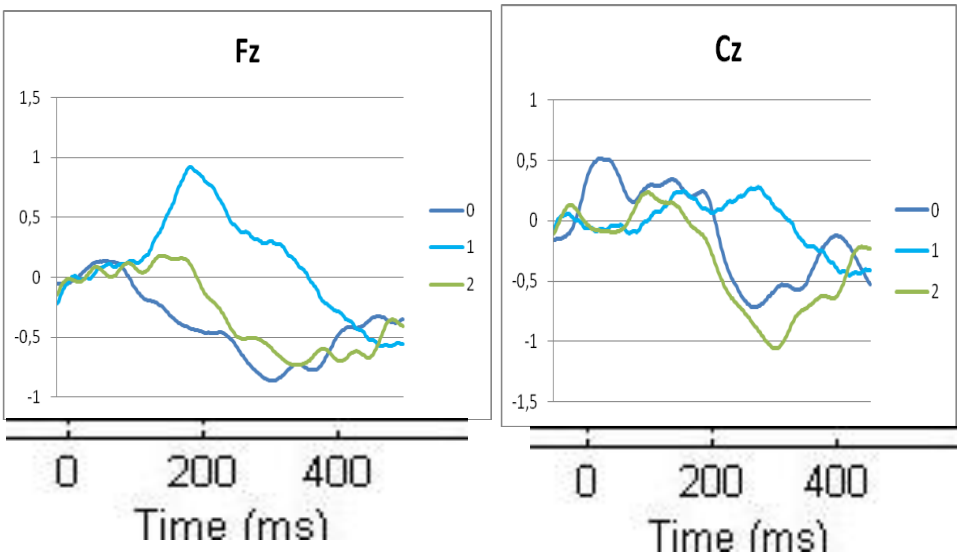
1000 Hz paradigm. An effect of group (p=.01) and time (p=.03) was found on Fz. Bonferroni's post hoc showed a statistical difference only between ELGA and LGA groups (p=.02). No statistical differences were found on Cz. Grand mean waveform on Fz and Cz is shown in Figure 9.1.

Fig. 9.1. Grand mean waveform on Fz and Cz sites in the three groups: ELGA (0), VLGA (1), LGA (2).



500 Hz paradigm. No statistical differences were found between groups and time windows on Fz and Cz (Figure 9.2). The examination of individual CAEP responses highlighted a high variability between responses.

Figure 9.2. Grand mean waveform on Fz and Cz sites



9.3.2 Behavioral developmental index at 12 months corrected age

No correlations were found between CAEPs and Griffith's sub-scales and total scores.

9.4 Discussion

In response to 1000 Hz stimuli, a wide positive waveform is recognizable in grand average of VLGA and LGA infants, while no response is recognizable in ELGA group (almost as grand average waveform). We did nott find the classical positive-negative complex recorded in near term infants (Graziani et al., 1974; Kushnerenko et al., 2002; Wunderlich et al., 2006), but a wide positive response. An explanation may be the short inter-stimulus interval used in our paradigm (600-900 ms).

The presence/absence of responses reflects significantly on differences between ELGA and LGA groups, being responses of VLGA group too variable to reach statistical significance.

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 (Bisiacchi et al., 2009). 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 been clarified yet (Novak et al., 1989; Suppiej, 2007), primary and secondary auditory cortices appear implicated in CAEP generation (Ponton et al., 2000); it suggests an alteration in cortical development underlying the differences in auditory processing between premature infants.

The suggestion that a cognitive dysfunction later in life can be predicted by abnormal auditory event related potentials recorded at birth and in follow up has been an interesting research hypothesis in learning disabilities (Leppa¨nen et al., 1997), autism (Lepisto 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).

The finding by Mikkola and colleagues (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, with a long lasting effect later in life.

We failed to find any correlation between CAEP and Griffiths developmental scores. However, the main development at one year of age is characterized by motor acquisitions, with other developing functions spinning around it. For this reason developmental index at this time is slightly predictive for later cognitive and neuropsychological development. Neurodevelopmental outcomes are difficult to

predict (Latal, 2009) and become evident later in life. Long term follow-up of premature infants recruited would be useful to clarify whether failure to develop any component of CAEP might be an early indicator of future neurodevelopmental disabilities.

The high variable responses to 500 Hz sounds may reflect a less developed auditory processing skills to lower frequencies. 500 Hz is a frequency very similar to those of human voice: it is possible that auditory overstimulation in the NICU due to nurse's and sanitary staff's talking has affected development of highly refractory neurons in the auditory cortex.

In conclusion, our results confirm previously reported differences in CAEP developmental trajectory between infants born at very low and extremely low gestational age. However, we were not able to identify correlation between CAEP and follow up at 12 months corrected age.

10. CONCLUSIONS

This work investigated early subtle brain electrical alterations in premature infants experiencing different developmental pathways and the association of these differences with later development. We therefore compared the EEG spectral power values and the CAEP recorded at the same post-conception weeks between infants born at different gestational ages; thus we correlated these neonatal data with behavioural development scores at 12 months corrected age.

At 35 post-conception weeks, relative spectral power values differed significantly between ELGA and LGA groups. Infants born at lower gestational ages had a higher amount of power in the δ frequency range, reflecting a delay in the relative reduction of lower frequencies occurring at this age or a protective rebound of slow activity. Although α and β were still very underrepresented at this time, an effect of gestational age was shown also on these less represented frequencies, with ELGA infants showing a lower amount of α and β spectral power.

The persistence of slow δ waves after 33-34 weeks of gestation is related to adverse outcomes (Hayakawa et al., 1997) and may reflect a developmental delay or the effect of long exposition to extrauterine stimulation on neural shaping processes. Higher frequency activities differ in morphology and localization from those recorded in adults and their functions are not well understood, but their importance as markers of development was demonstrated (Watanabe, 1992; Scher et al., 1994). Several specific high frequency features are known as a marker of electroencephalographic maturation. Therefore, an objective evaluation of the corresponding spectral range will be useful to evaluate brain maturation in preterm infants.

The subgroup of premature infants followed from 35 to 40 post-conception weeks presented a coherent developmental pattern as they reached 40 post-

conception age, with a decrease in δ activity and a mild increase in higher frequency (not significant); by comparing these infants to a group of full-term infants, no significant differences in spectral power values were found. Although the sample was very small (10 premature *vs* 10 full-term infants), it is possible that earlier neurophysiological evaluations allowed to photograph brain functionality during a critical window. Subtle alterations present in brain activity at any stage may not be more evident later: EEG disturbances are more likely to be documented if recordings are obtained more closely to the time when the disturbance to brain function occurs (Scher et al., 1995). Abnormal patterns become progressively less demonstrable during the convalescent period.

Data from CAEP are in agreement with previous data (Bisiacchi et al., 2009) and in line with the results coming from spectral analysis. In response to 1000 Hz tones no waveforms became evident on Fz in ELGA infants, while LGA presented a wide and slow positive response. VLGA group waveform resembled that of LGA group, but characterized by high variability.

The lack of responses to stimulation in ELGA group as recorded in CAEP may reflect slower recovery processes in response to the relatively short inter-stimulus interval; in any case, more immature functionally neural networks seem to characterize the brain substrate of these infants.

All the reported differences remained speculative if not associated with alterations in cognitive functioning at later age (particularly after children enter school). The preliminary data on those infants attaining 12 months of corrected age confirmed the sensivity of neurophysiological techniques in highlight alterations in brain functioning at early stage of development. Until about two years of age, development of premature infants is still delayed and a correction for gestational age is requested to obtain normative values on developmental scale scores, a routine

clinical practice. Although the scores of our infants resulted adequate for corrected age on the whole, children born at lower gestational age performed worse in motor subscales (gross-motor and manual ability). More interestingly, relative spectral power values correlated with developmental index scores according to a coherent pattern: a higher amount of δ and a lower amount of β and α resulted associated with poor relational skills and personal self autonomies. Despite the small sample, these results appear particularly meaningful in the light of the reported high prevalence of psychiatric and behavioral disorders in ex premature children and adolescents (Bhutta et al., 2002).

In conclusion, neurophysiological methodologies, easily performed bedside, provide information about brain functions and help to identify possible factors which interfere with normal brain development. The potential of neurophysiological methodologies may be the fact that the use of signal analytic features not readily identified at visual inspection may provide a useful indicator of good prognosis or poor developmental outcomes.

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