

Electroencephalographic functional connectivity in extreme prematurity: a pilot study based on graph theory

Cite this article as: Elisa Cainelli, Maria Grazia Di Bono, Patrizia S. Bisiacchi and Agnese Suppiej, Electroencephalographic functional connectivity in extreme prematurity: a pilot study based on graph theory, *Pediatric Research* doi:10.1038/s41390-019-0621-3

This Author Accepted Manuscript is a PDF file of an unedited peer-reviewed manuscript that has been accepted for publication but has not been copyedited or corrected. The official version of record that is published in the journal is kept up to date and so may therefore differ from this version.

Terms of use and reuse: academic research for non-commercial purposes, see here for full terms. <https://www.nature.com/authors/policies/license.html#AAMtermsV1>

Author accepted manuscript

Cover Page

Title: Electroencephalographic functional connectivity in extreme prematurity: a pilot study based on graph theory

Running Title: Connectivity in premature infants

Elisa Cainelli¹, Maria Grazia Di Bono², Patrizia S. Bisiacchi^{3,4} and Agnese Suppiej^{*1,5}

¹*Pediatric University Hospital, via Giustiniani n. 3, Padua, Italy.*

²*Department of General Psychology, University of Padua, via Venezia n. 8, Padua, Italy.*

³*Lifespan Cognitive Neuroscience Laboratory (LCNL), Department of General Psychology, University of Padua, via Venezia n. 8, Padua, Italy. Padova Neuroscience Center, PNC*

⁵*Department of Medical Sciences, Pediatric Section, University of Ferrara, Italy.*

Elisa Cainelli and Maria Grazia Di Bono contributed equally to the work

***Corresponding Author:**

Agnese Suppiej MD, PhD, Department of Medical Sciences, Pediatric Section,

University of Ferrara,

Via Aldo Moro 8, 44124 Cona, Ferrara, Italy

Tel 0039-3473677013

Fax 0532 23 78 48 E-mail: agnese.suppiej@unife.it

Disclosure statement. None of the authors has potential conflicts of interest to be disclosed.

Statement of Financial support. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Category of Study: clinical research article.

Author Contributions:

Elisa Cainelli: collaborate to write the first draft of the manuscript, contributed to conception and design of the study; performed the recordings, interpreted the data; approved the final version to be submitted.

Maria Grazia Di Bono: collaborate to write the first draft of the manuscript. Contributed to design of the study; performed the data analyses; approved the final version to be submitted.

Patrizia Silvia Bisiacchi: collaborate to interpretation of the data, reviewed critically the manuscript, and approved the final version to be submitted.

Agnese Suppiej: collaborate to conception, design of the study and interpretation of data; reviewed critically the manuscript and wrote the final version of the manuscript.

Abstract

Background. Connectivity studies based on functional magnetic resonance imaging (MRI) provided new insights in neonatal brain development but cannot be performed at bedside in the clinical setting. The electroencephalogram (EEG) connectivity has been less studied, particularly using the new approach based on graph theory. This study aimed to explore the functional EEG connectivity using graph theory analysis at an early post-conception age in extremely premature and late-preterm babies, free of medical complications and overt brain damage.

Methods. Sixteen neonates (8 extremely low gestational age -ELGA- and 8 late-preterm infants), having both performed multichannel EEG recordings at 35 weeks post conception, were recruited in a single tertiary-level neonatal intensive care unit and well-baby nursery, respectively. Global (i.e., small-worldness) and local (i.e., clustering and strength) connectivity measures were calculated on a single-subject-connectivity-matrix of EEG data.

Results. Both ELGA and late-preterm infants showed small-worldness organization at 35 weeks post-conception. The ELGA group had the strength parameter of the theta frequency band lower in the right than in the left hemisphere. This asymmetry did not emerge in the late-preterm group. Moreover, the mean strength parameter was significantly greater in the right hemisphere in the late-preterms than in the ELGA group.

Conclusion. EEG connectivity measures could represent an index of left-to-right maturation and developmental disadvantage in extremely preterm infants.

INTRODUCTION

Preterm infants are at developmental risk, even in the absence of overt brain damage. The causes are not yet well understood, but it has been speculated that prolonged extra-uterine experience and/or medical and environmental conditions leading to premature birth may interfere with brain maturation in a crucial phase (1,2). Research from many fields of neonatal medicine is increasingly focusing on structural and functional pathways reshaping in the last trimester. Electroencephalographic (EEG) (1,3,4) and magnetic resonance imaging (MRI) (5) studies already revealed subtle and/or transient abnormalities of brain development in preterm infants having attained term equivalent age. Furthermore, in a previous study of infants born at extremely low gestational age, we found that, as early as 35 weeks post conception, failure to develop age specific patterns of EEG spectral activity, might represent a neurodevelopmental risk, in the absence of overt brain damage and neurological risk factors other than prematurity (6).

In the last decade, connectivity studies based on diffusion tensor imaging, functional MRI (fMRI), EEG and magnetoencephalography provided new insight in neonatal brain functioning by comparing preterm infants with healthy infants born at term (7-11). EEG resting-state data provide insight into functional brain connectivity through the patterns of different brain oscillations. The study of EEG networks is complementary to fMRI and has the advantage of being available at bedside in the neonatal intensive care units. One other advantage of using electrical signals is that they allow exploring networks with spatial and temporal dynamics that functionally are not completely overlapping to resting state-fMRI (12) providing further information on the underlying neurophysiological mechanisms.

Research on neonatal EEG connectivity in extreme prematurity provided important information on development of brain networks, particularly on maturation of short-distance and long-distance brain connections (13-16).

The increasingly high number of data-sets on brain function, lead to the need of a new, multidisciplinary approach to the study of complex systems, known as complex network analysis, considering that the human brain as an extremely complex system of interacting physical and functional units, ranging from single neurons to complex networks (17). Complex network analysis has its origins in the mathematical study of networks, known as graph-theoretical analysis. Graph-theoretical analysis offers a mathematical language to describe segregation and integration properties of brain functioning. It analyzes functional interactions among brain areas at a topological level (18). The brain is modeled as a graph in which each node (e.g., a brain area or a sensor channel) is linked to all the other nodes within the graph, through edges that are weighted by some measure of correlation.

The fMRI studies investigating development of functional connectivity highlighted differences between early premature and term infants, using graph-theoretical analysis, but comparable studies using the EEG are lacking (19).

The present exploratory study aimed to assess brain function in preterm infants by means of EEG connectivity measured through the graph theory analysis. In order to highlight maturational effects and minimize the influence of confounding variables, we focused on neonates without medical complications and overt brain damage.

METHODS

Participants

Sixteen premature infants (8 ELGA infants, range 23+2 –27+6 weeks gestation, and 8 late-preterm infants, range 34 – 35+2 weeks gestation) were recruited respectively from the Neonatal Intensive Care Unit and from the well-baby nursery at the Pediatric University Hospital of Padua, Italy.

Exclusion criteria were: intrauterine growth restriction (defined as estimated fetal weight below the 10th percentile and umbilical artery pulsatility index greater than 2 standard deviations (20), cranio-facial malformations, genetic syndromes, clinical evidence of neonatal encephalopathy, brain ultrasound evidence of intraventricular hemorrhage (21) or periventricular cystic leukomalacia (as defined by De Vries et al. (22), occurrence of seizures. Furthermore, infants treated with drugs (e.g., sedatives) affecting the central nervous system and/or with abnormal EEGs, evaluated by the same neurophysiologist (A.S.), by visual inspection, were excluded from the study.

Clinical data are summarized in Table 1.

The hospital ethical committee (Comitato Etico per la Sperimentazione Clinica dell’Azienda Ospedaliera di Padova) approved the study. Parents gave informed consent.

Data acquisition

The EEG was recorded following International System of electrode placement and international guidelines for neonates as previously described.⁶ Fp1, Fp2, Fz, C3, Cz, C4, T3, T4, O1 and O2 recording electrodes referred to the linked earlobes were used. The EEG was recorded using a Galileo system (EB Neuro/Florence, Italy). The digital EEG signal was sampled at 512 Hz and stored on a hard disk. The band-pass filtering was 0.5–20 Hz. An

artifact rejection routine was used to exclude segments of the EEG in which the voltage in any channel exceeded 1000 μV .

All recordings took place between 10.30 and 12 h a.m. a time slot in which no medical or feeding procedures were carried out. For offline analysis, five minutes of artifact free EEG trace recorded in active sleep were selected from at least one-hour recording. We choose active sleep state because it represents predominantly neocortical activity (23).

Data analysis

Network construction

The functional brain network was constructed using a single-subject-connectivity-matrix approach, as suggested by Langer and colleagues (24). Then, we used the brain connectivity toolbox (17) for extracting the network measures.

For each infant of the two groups: we applied a linear detrend to each channel signal, by removing its mean value; then, for each of the considered frequency bands (delta [0.5 - 4 Hz]; theta [4 - 8 Hz]; alpha [8 - 12 Hz] ; beta[12 - 20 Hz]), we computed the adjacency matrix by using the magnitude square coherence (MSC) as a measure of phase synchronization among each channel pair.

The MSC is the ratio of the squared magnitude of the cross-power spectrum between two signals to the product of their individual power spectra. Specifically, the MSC estimate is a function of frequency with values between 0 and 1 that indicates how well the signal x corresponds to the signal y at each frequency. The MSC is a function of the power spectral densities, $P_{xx}(f)$ and $P_{yy}(f)$, of x and y , and the cross power spectral density, $P_{xy}(f)$, of x and y :

$$C_{xy}(f)=|P_{xy}(f)|^2 / P_{xx}(f)*P_{yy}(f)$$

In particular, we used the MATLAB function ‘mscohere’ for computing the MSC, which estimates the magnitude-squared coherence function (25) using Welch's overlapped averaged periodogram method (see 26). We used sections of 2 seconds with 50% of overlap averaged with Hanning window, as parameters to the ‘mscohere’ function, for computing the cross spectral density and the power spectral densities of each signal in the pair. Then we used the proportional thresholding method for constructing each graph starting from each adjacency matrix: we ranked the connections (i.e., the coherence values) in a descendent order and selected a certain percentage of the top ranked connections, expressed by a specific threshold. The selection of the network threshold for the construction of the final graphs was performed by adopting a procedure based on controlling the small-world parameter as function of the network threshold (see 24).

The problem of thresholding in graph theoretical network analysis is still an open question, but some suggestions have been reported in a methodological study by Langer and colleagues (24), where different possibilities can be chosen. In particular, using unthresholded weighted connectivity matrices it is possible to statistically test the network parameters. Thresholded networks, however, exhibit a clearer small-world topology, because the noise of the data is reduced by the thresholding procedure]. In some studies (e.g., 27), the authors presented a further possibility: they first determined the larger threshold, which exhibited the best small-world topology and then used this threshold. Small-world organized networks are characterized by a clustering coefficient C that is higher than the C of a randomly organized network (C_{random}), and a short characteristic path length L that is similar to that of an equivalent random network (L_{random}) (27). Formally, small-world networks show a ratio c defined as $C_{\text{real}}/C_{\text{random}}$ that has to be greater than 1 and a ratio k defined as $L_{\text{real}}/L_{\text{random}}$ that has to be about 1 (27). A high c reflects a high level of local neighborhood

clustering within a network, and a short travel distance k expresses a high level of global communication efficiency within a network (18). For each subject, the small-world indices were derived from the comparison of the real (measured) network with a random network including the same number of nodes, edges and degree distribution of the real network. Random networks were constructed by using the function “randmio_und” contained in the brain connectivity toolbox of Rubinov and Sporns (17). This function randomizes an undirected weighted network, while preserving the degree, but not the strength distribution. The best small-world topology in (24) has been defined by a high c and a $k \approx 1$. In our study, this could be automatically translated into the selection of the larger threshold that guarantees a small world parameter > 1 , and can allow to maintain a connected graph (with the more salient information) and discard the more defragmented graphs (containing spurious connections) at the lower thresholds.

For each infant at each frequency band, we started from the adjacency matrix and used the proportional thresholding method for constructing the corresponding cortical connectivity network: we ranked the connections (i.e., the MSC values) of each adjacency matrix in a descendent order and selected a certain percentage of the top ranked connections, expressed by a specific proportional threshold (i.e., [0.2 – 0.8] with step of 0.1). In particular, we used the function “threshold_proportional” of the brain connectivity toolbox of Rubinov and Sporns (17): this function thresholds the connectivity matrix by preserving a proportion p ($0 < p < 1$) of the strongest weights. All the other weights, and all the weights on the main diagonal (self-self-connections) are set to 0. Successively, we computed the small world parameter (using the formulation proposed by Humphries and Gurney (28), as implemented in the brain connectivity toolbox of Rubinov and Sporns (17)) at each considered threshold, and selected the larger threshold (i.e., in our case 50% of the top ranked connections, as

reported in the Results section) that preserved the small world network topology (i.e., the mean small-world parameter > 1) and maintained a connected graph, discarding the more defragmented graphs at the lower thresholds.

Connectivity measures

We adopted some of the most commonly used measures of global/local connectivity. Specifically, as a global measure we selected the small-world parameter for the network construction. Crucially, small-world parameter was used for thresholding the adjacency matrices and obtaining the weighed graphs (24). *Small-worldness* (i.e., small-world parameter > 1) is a property characterizing graphs with dense local clustering and relatively few long-range connections, and it can globally account for both specialized (segregated) and distributed (integrated) information processing, revealing a more general different level of efficiency in information processing between groups. In order to better distinguish between segregation and integration properties within brain networks, we selected two local connectivity measures: (i) *Clustering* coefficients, which are locally computed as the fraction of triangles around each individual node; (ii) *Strength*, which is computed for each node of the network as the sum of the weights on its connections. The clustering coefficient reflects the prevalence of clustered connectivity around individual nodes, and roughly corresponds to an index of segregation and specialization. Strength in functional networks is an index of integration and synchronization of brain activity. Both clustering and strength measures were computed using the brain connectivity toolbox of Rubinov and Sporns (17), on the normalized weighted graphs obtained after thresholding. Specifically, after thresholding, we use the function “weight conversion” (used with the parameter option “normalize”), contained in the brain connectivity toolbox of Rubinov and Sporns (17) for normalizing the cortical connectivity network. This function scales all weight connections to the range [0,1]

by dividing the connection values to the maximal weight, and should be done prior to computing some network parameters, such as the clustering coefficient. Indeed, since network measures strictly depend on the mean of the weighted connections, weighted graphs obtained after thresholding need to be normalized in order to perform statistical analysis on the extracted measures.

Statistical analysis

For the global measure (i.e., small worldness) we performed a t-test for independent samples (i.e., ELGA group vs late-preterm group). Due to the small sample size (i.e., 8 participants per group), we also performed a nonparametric t-test (i.e., both the Mann-Whitney and Welch tests). The Mann-Whitney test, the most general one, means that we do not need any distributional assumptions about the probability distribution that the data comes from. The Welch test adds the assumption that the two groups come from a normal distribution, but the variance in the two groups can be different. At a local level (i.e., strength and clustering), we investigated whether potential distinctions, in terms of connectivity, between ELGA and the late-preterm group could be modulated by hemispherical differences. For each local measure (i.e., strength parameter and clustering coefficient), we excluded the central channels (i.e., Fz and Cz) and averaged the values obtained for the channels located on the left and right side of the scalp. Then, we performed an analysis of variance on these measures, using the hemisphere (left vs right) as a within factor and the group (ELGA group vs late-preterm group) as between factor. In order to explore interactions, we also performed nonparametric t-tests (i.e., Mann-Whitney and Welch tests for testing the group differences and the Wilcoxon ranked test for testing hemispheric differences).

RESULTS

Network construction

We chose a threshold of 0.5 in all the frequency bands that is the maximum threshold guaranteeing small-worldness (i.e., the small-world parameter was upon the value of one).

Figure 1 shows the values of the small-world parameter as a function of the threshold. Thus, for each subject we constructed a graph of the same density (i.e., the number of connections).

Figure 2 (panel a) shows an example of the coherence graphs (averaged across subjects for each group) obtained after pruning the adjacency matrices at a threshold of 0.5 in the theta band.

Global coherence-graph measures

No significant differences in small-worldness emerged between ELGA and late-preterm groups in all the frequency bands (all $t_s \leq 1.27$, all $p_s > .23$). The nonparametric t-tests confirmed the results (e.g., in the theta band: Welch $t(13.74) = -0.77$, $p = 0.45$; Mann-Whitney $t = 26$, $p = 0.57$).

Local coherence-graph measures

Results on strength parameter in theta band showed a significant interaction ($F(1,14) = 6.19$, $p = .026$, $\eta_p^2 = .31$), whereas no main effect of hemisphere ($F = 1.09$, $p = .31$) and group ($F = 1.36$, $p = .26$) emerged. T-tests showed that only for the ELGA group there was a significant hemispheric difference ($t(7) = 2.44$, $p = .045$), which was explained by a reduced strength parameter in the right ($M = 1.94$, $SEM = .13$) rather than the left ($M = 2.48$, $SEM = .27$) hemisphere. This asymmetry did not emerge, instead, in the late-preterm group ($t = -1.05$, $p = .33$). Moreover, no group effect emerged in the left hemisphere ($t = .46$, $p = .65$), whereas,

in the right hemisphere the mean strength parameter was significantly greater in the late-preterm group ($M = 2.57$, $SEM = .13$) than in the ELGA group ($M = 1.94$, $SEM = .13$) ($t(14) = 2.84$, $p = .013$), as shown in Figure 2 (panel b and c). Additional nonparametric t-tests confirmed and strengthened these results. Wilcoxon ranked tests showed that only for the ELGA group there was a significant hemispheric difference ($t = 33$, $p = .039$), whereas no significant difference emerged for the late pre-term group ($t = 14$, $p = .64$). Moreover, only in the right hemisphere the mean strength parameter was significantly greater in the late-preterm group than in the ELGA group (Welch test: $t(12.51) = 2.84$, $p = .014$; Mann-Whitney test: $t = 8$, $p = .01$). No group difference emerged in the left hemisphere (Welch test: $t(10.29) = .46$, $p = .65$; Mann-Whitney test: $t = 34$, $p = .88$).

We found no significant differences on clustering coefficient in all the frequency bands (all $F_s \leq 1.7$, all $p_s \geq 0.29$).

DISCUSSION

Our research is a cross-sectional exploratory study applying the graph theory to the EEG analysis of a small group of preterm infants without neurological risk factors, born at different gestational ages but all tested at 35 weeks post conception. We found a small-worldness organization both in ELGA and late-preterm infants, and we found no differences in small-worldness organization between groups. By considering local measures, only the ELGA group showed a hemispheric asymmetry in the strength parameter in the theta band, characterized by a lower strength parameter in the right hemisphere, suggesting reduced functional integration in this hemisphere. This result was also reflected on mean strength parameter, significantly greater in the right hemisphere of the late-preterm group compared to the ELGA group. This asymmetry between hemispheres is in line with previous neuroimaging, neurophysiology and post-mortem studies (29).

Thirty-five-week gestation is a pivotal time for the cerebral development, as shown by post-mortem and neurophysiological studies (2). Our findings suggest that as early as 35-week post-conception, developmental trajectory of extremely preterm infants is different from their near-term peers.

Previous research showed differences in functional connectivity between premature infants as they reach term and healthy full-term infants, although with some conflicting results (7,9-11,30).

Given that EEG represents the synchronous activity of neurons arranged perpendicular to the surface of the cerebral cortex, the methodology do not allow not allow a direct measure of subcortical and white matter interconnections. However, several EEG algorithms offer interesting insight about how cortical areas work together in networks and, thanks to the high temporal resolution, the link with the resulting cognitive processes becomes more suitable.

Among newly developed analysis techniques, graph theory is very interesting given its possibility to provide information about global and local level of the cerebral organization (for details, see 17). All these graph metrics provide evidence of both segregation (e.g., clustering) and integration (e.g., strength) properties of the graph. An emergent property of many complex networks in nature is the “small-world” topology, which is in-between regular (i.e., each node is linked only to its neighbors) and random (i.e., each node is randomly connected to all the other nodes) graph topologies. Small-worldness characterizes graphs with dense local clustering and relatively few long-range connections, which is an appealing property, because it can globally account for both specialized (segregated) and distributed (integrated) information processing.

It has been suggested that the prevalence of small-world networks in biological systems may reflect the evolutionary advantage of a greater sustainability of system performance under perturbations (31). Our findings on the small-worldness suggest that this important property emerges at an early developmental phase and it is independent from gestational age. Studies in the child and adult brain suggest that the small-world architecture may be the hallmark feature of macroscopic cortical connectivity (18). A small-world architecture has been demonstrated in neonates as early as 30 weeks gestation using resting-state fMRI (8,32) and other fMRI connectivity approaches (7,11) and is also consistent with results obtained using other analysis methods applied to the EEG (13).

The majority of graph studies investigating cross-sectionally the development of functional connectivity in premature infants used fMRI data, literature lack of studies using EEG. Although brain imaging may reveal both structure and function, EEG provides complementary information on much faster time scales (milliseconds) combined with a relatively easier assessment compared to neuroimaging. One other advantage of using

electrical signals is that they allow exploring networks exhibiting spatial and temporal dynamics that functionally are not completely overlapping to their resting state-fMRI counterparts (12). A further advantage of EEG based network analysis, with respect to fMRI, is that it offers additional information on the underlying neurophysiological mechanisms by the evaluation of the different EEG frequency bands.

Regarding local coherence measures obtained in our study, the mean strength parameter in the theta band was significantly greater in the right hemisphere of late-preterm group than that of ELGA group. Although several studies documented differences between preterm infants and full-term neonates, no study investigated cross-sectionally the functional connectivity of infants born extremely premature earlier than at attainment of term age. Our findings suggest that as early as 35-weeks post conception, developmental trajectory of extremely preterm infants without neurological risks, could be different from their late-preterm peers, in line with previous studies (3,6). Using graph analysis of fMRI data, van den Heuvel and colleagues (32) found patterns of global and local connectivity similar to our results. In their study, disruptions in network structure were shown to arise from changes secondary to the early emergence of rich-club organization, such as the increase in local cortico–cortical connectivity.

In our study, differences in strength parameter between ELGA and late-preterm groups were statistically significant only in the theta frequency range. It has been speculated that the increase of theta band frequencies may predate and facilitate the formation of long-range functional networks (33) and thalamo-cortical connections, possibly associated to development of neocortical and hippocampal networks (34). Changes in the theta band power have been implicated in the development of complex frequencies in human medial frontal cortex (13,33). Abnormalities in theta activity, at an early stage, could trigger a cascade of

alterations in the development of high frequencies, occurring later in life. Our findings are particularly interesting considering that prematurity per se is associated with higher rates of neurodevelopmental disorders, such as attention deficit hyperactivity disorder and autism, which have both been recently characterized as disorders of connectivity (35).

The study of brain maturation on premature infants at very early ages is challenging. Given the high vulnerability of premature brains, the recruitment of infants born at extremely low gestational age without neurological risk factors other than prematurity is very demanding. Premature infants are at risk for several complications of prematurity with different effect on the EEG depending on the gestational age and the clinical condition, making it difficult to compare results from different studies. In fact, several studies did not exclude children with comorbidities (for example 19), introducing important confounding variables in the study of developmental trajectories.

Furthermore, the EEG may be technically challenging when cooperation is lacking and in the intensive care setting, due to increased presence of artifacts. One other problem is the small surface for electrode placement (both for the reduced head circumference and for the possible presence of medical aids) that decreases the possibility of measuring many scalp sites. Beside all these limitations, the EEG still represent a very useful tool for studying developmental trajectories in prematurity, a unique window on the brain at work during the early phases, which parallel basic science studies of histological and neurochemical processes and neuroimaging studies (2). The EEG network studies provided a variety of analysis techniques and advancements are continuously evolving. Furthermore, the enormous advancements in the evaluation and analysis of neonatal EEG signals, combined with progress in basic sciences is providing a theoretical framework for better understanding mental health disorders of later life (2).

The present study has some intrinsic methodological limitations as well as some strengths. We used coherence as a correlation measure for constructing the adjacency matrices. We acknowledge that coherence is a linear measure of correlation among channel pairs and it can be sensitive to volume conduction (see for review 36). However coherence, is considered a standard measure of cortical networks at the level of both scalp and source space (37), and numerous studies performed connectivity analysis using coherence measures, for example (13,38-39). In our study, we controlled for these coherence limitations through specific methodological choices. Firstly, we used network measures instead of the direct measure of coherence. Computing other connectivity measures such as strength/clustering, lead us to focus on the nodes (i.e., channels) and their interaction with the neighbor nodes (channels), rather than the individual links (i.e., coherence among channel pairs), and to take into account more informative properties of brain functioning such measures of segregation/integration (indexed respectively by strength/clustering measures). Moreover, in the statistical analyses, we focused on global network metrics (i.e., small world parameter), and on hemispheric asymmetries by averaging the local/nodal network measures (i.e., strength and clustering coefficient) across the channels within each hemisphere. In this way, the coherence sensitivity to volume conduction was partially controlled. One other intrinsic methodological limitation of this study was that the network construction was based on the EEG signal registered from eight channels, due to the small head circumference of the infants at 35 PCA. We choose a traditional EEG apparatus, which allowed us to place only eight channels given the small head size, in order to limit the use of invasive procedures and to record at bedside before discharge. A similar EEG methodology was successfully used by other authors previously in order to investigate cerebral connectivity in premature infants (13,14).

One other possible limitation of the study is the choice of the filter band-pass that do not allow analyzing high EEG frequencies. We choose to apply a filter of 0.5-20Hz because previous literature showed that the maturation of intracortical interneuronal circuits and of inhibitory neurotransmission - processes that span from full-term age further into infancy - are required before cortical networks become able to generate robust higher frequency activity (beta and gamma). Therefore, most of the EEG power in the premature infants comes from the lowest frequencies.

However, the major limitation of the study is the small sample size. Although this limitation, results appear in line with studies based on other EEG analysis techniques or fMRI (7-11) and show that the technique could be used, adopting the standard clinical setting of neonatal EEG used at bedside in NICU. Considering the small number of recruited neonates, results should be, however, taken carefully. The value of the present study was the focus on a very homogeneous group of preterm infants without medical complications, brain imaging abnormalities and other comorbidities, born at extremely low gestational age. We suggest the possibility of analyzing their neonatal EEG by means of complex mathematical models specifically the graph-theoretical analysis, to evaluate early development of cortical networks and to distinguish the developmental trajectory of neonates born at extremely low gestational ages from those born near term.

In conclusion, the application of the graph theory on preterm EEG analysis offers a useful tool for exploring functional connectivity during the early phases of brain development. Global coherence-graph measures suggest a small-world organization in the network topology in both extremely preterm and late-preterm infants, already at 35 weeks post conception. Local coherence-graph measures were able to detect, at comparable age post conception, subtle differences in connectivity between infants born late-preterm and those

born before the 28th gestational week. Further studies on larger populations are needed to confirm these results.

Disclosure statement. None of the authors has potential conflicts of interest to be disclosed.

Statement of Financial support. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Suppiej A, Cainelli E, Cappellari A, et al. Neonatal Cortical Auditory Evoked Potentials Are Affected by Clinical Conditions Occurring in Early Prematurity. *J Clin Neurophysiol* 2015;32:419-23. doi: 10.1097/WNP.0000000000000182.
2. Suppiej A, Cappellari A, Cainelli E. Clinical neurophysiology in preterm infants: a window on early phases of brain development, in: Lawson PN, McCarthy EA (eds.), *Pediatric Neurology, Series: Neuroscience Research Progress*. Nova Publisher, ISBN:978-1-61470-161-https://www.novapublishers.com/catalog/product_info.php?products_id=26363 (accessed May 2012).
3. Bisiacchi PS, Mento G, Suppiej A. Cortical auditory processing in preterm newborns: an ERP study. *Biol Psychol* 2009;82:176–85. doi: 10.1016/j.biopsycho.2009.07.005.
4. Scher MS, Jones BL, Steppe DA, et al. Functional brain maturation in neonates as measured by EEG-sleep analyses. *Clin Neurophysiol* 2003;114:875–82.
5. Ball G, Boardman JP, Aljabar P, et al. The influence of preterm birth on the developing thalamocortical connectome. *Cortex J Devoted Study Nerv Syst Behav* 2013;49:1711–21. doi: 10.1016/j.cortex.2012.07.006.
6. Suppiej A, Cainelli E, Cappellari A, et al. Spectral analysis highlight developmental EEG changes in preterm infants without overt brain damage. *Neurosci Lett* 2017;649:112-5. doi: 10.1016/j.neulet.2017.04.021.
7. Doria V, Beckmann CF, Arichia T, et al. Emergence of resting state networks in the preterm human brain. *PNAS* 2010;107:20015–20. doi: 10.1073/pnas.1007921107.

8. Fransson P, Åden U, Blennow M, et al. The functional architecture of the infant brain as revealed by resting-state fMRI. *Cereb Cortex* 2011;21:145-54. doi: 10.1093/cercor/bhq071.
9. Ball G, Aljabar P, Zebari S, et al. Rich-club organization of the newborn human brain. *PNAS* 2014;111:7456-61. doi: 10.1073/pnas.1324118111.
10. Toulmin H, Beckmann CF, O’Muircheartaigh J, et al. Specialization and integration of functional thalamocortical connectivity in the human infant. *Proc Natl Acad Sci* 2015;112:6485–90. doi: 10.1073/pnas.1422638112.
11. Smyser CD, Snyder AZ, Shimony JS, et al. Resting-State Network Complexity and Magnitude Are Reduced in Prematurely Born Infants. *Cereb Cortex* 2016;26:322–33. doi: 10.1093/cercor/bhu251.
- 12 Omidvarnia A, Metsäranta M, Lano A, et al. Structural damage in early preterm brain changes the electric resting state networks. *Neuroimage* 2015;120:266-73. doi: 10.1016/j.neuroimage.2015.06.091.
- 13 Meijer EJ, Hermans KHM, Zwanenburg A, et al. Functional connectivity in preterm infants derived from EEG coherence analysis. *Eur J Paediatr Neurol* 2014;18:780–9. doi: 10.1016/j.ejpn.2014.08.003.
- 14 De La Cruz DM, Mañas S, Pereda E et al. Maturation changes in the interdependencies between cortical brain areas of neonates during sleep. *Cereb Cortex* 2007;17:583–90.
- 15 Scher MS, Steppe DA, Banks DL. Prediction of lower developmental performances of healthy neonates by neonatal EEG-sleep measures. *Pediatr Neurol* 1996;14:137–44.
- 16 Tymofiyeva O, Hess CP, Ziv E et al. Towards the “baby connectome”: Mapping the structural connectivity of the newborn brain. *PLoS ONE* 2012;7:e31029.
- 17 Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. *Neuroimage* 2010;52:1059-69. doi: 10.1016/j.neuroimage.2009.10.003.

- 18 Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci* 2009;10:186–98. doi: 10.1038/nrn2575.
- 19 Tokariev A, Stjerna S, Lano A, Metsäranta M, Palva JM, Vanhatalo S. Preterm Birth Changes Networks of Newborn Cortical Activity. *Cerebral Cortex* 2019;29: 814–826.
20. Cosmi E, Visentin S, Fanelli T, et al. Aortic intima media thickness in fetuses and children with intrauterine growth restriction. *Obstet Gynecol* 2009;114:1109-14. doi: 10.1097/AOG.0b013e3181bb23d3.
21. Volpe JJ. Intracranial haemorrhage, in: Volpe JJ (ed), *Neurology of the Newborn* Philadelphia: WB Saunders Company, 2000, pp. 428-93.
22. De Vries LS, Eken P, Dubowitz LMS. The spectrum of leukomalacia using cranial ultrasound. *Behav Brain Res* 1992;49:1-6.
23. Steriade M, Gloor P, Llinás RR, et al. Mesulam, Report of IFCN Committee on Basic Mechanisms, Basic mechanisms of cerebral rhythmic activities. *Electroencephalogr Clin Neurophysiol* 1990;76:481-508.
24. Langer N, Pedroni A, Jäncke L. The problem of thresholding in small-world network analysis. *PloS one* 2013;8:e53199. doi: 10.1371/journal.pone.0053199.
25. Kay SM. *Modern Spectral Estimation*. Englewood Cliffs, NJ: Prentice-Hall, 1988.
26. Rabiner LR, Gold B. *Theory and Application of Digital Signal Processing*. Englewood Cliffs, NJ: Prentice-Hall, 1975.
27. Sporns O, Zwi JD. The small world of the cerebral cortex. *Neuroinformatics* 2004;2(2):145-162.
28. Humphries MD, Gurney K. Network ‘small-world-ness’: a quantitative method for determining canonical network equivalence. *PloS one* 2008;3(4):e0002051.

29. Mento G, Suppiej A, Altoè G, et al. Functional hemispheric asymmetries in humans: Electrophysiological evidence from preterm infants. *Eur J Neurosci* 2010;31:565-74. doi: 10.1111/j.1460-9568.2010.07076.x.
30. Grieve PG, Isler JR, Izraelit A et al. EEG functional connectivity in term age extremely low birth weight infants. *Clin Neurophysiol* 2008;119:2712–20.
31. Salvador R, Suckling J, Coleman MR, et al. Neurophysiological architecture of functional magnetic resonance images of human brain. *Cereb Cortex* 2005;15:1332–42.
32. Van Den Heuvel MP, Kersbergen KJ, De Reus MA, et al. The Neonatal Connectome During Preterm Brain Development. *Cereb Cortex* 2015;25:3000-13. doi: 10.1093/cercor/bhu095.
33. Canolty RT, Knight RT. The functional role of cross-frequency coupling. *Trends Cogn Sci* 2010;14:506–55. doi: 10.1016/j.tics.2010.09.001.
34. Corner MA, van Pelt J, Wolters PS, et al. Physiological effects of sustained blockade of excitatory synaptic transmission on spontaneously active developing neuronal networks—an inquiry into the reciprocal linkage between intrinsic biorhythms and neuroplasticity in early ontogeny. *Neurosci Biobehav Rev* 2002;26:127–85.
35. Uddin LQ, Supekar K, Menon V. Reconceptualizing functional brain connectivity in autism from a developmental perspective. *Front Hum Neurosci* 2013;7:458. doi: 10.3389/fnhum.2013.00458.
36. van Diessen E, Numan T, van Dellen E et al. Opportunities and methodological challenges in EEG and MEG resting state functional brain network research. *Clin Neurophysiol* 2015;126(8):1468-81. doi: 10.1016/j.clinph.2014.11.018.

37. Bowyer SM. Coherence a measure of the brain networks: past and present. *Neuropsychiatric Electrophysiol* 2016;2(1):1. doi: 10.1186/s40810-015-0015-7.
38. Myers MM, Grieve PG, Stark RI et al. Family Nurture Intervention in preterm infants alters frontal cortical functional connectivity assessed by EEG coherence. *Acta Pædiatrica* 2015;104:670–677. doi:10.1111/apa.13007.
39. Peters JM, Taquet M, Vega C et al. Brain functional networks in syndromic and non-syndromic autism: a graph theoretical study of EEG connectivity. *BMC Medicine* 2013, 11:54.

Figure legends

Figure 1. Threshold selection for graph construction. Mean small-worldness plotted as a function of the network threshold. The threshold of 0.5, indicated by the black circle, was selected as the maximum threshold for which the small-world parameter is above the value of one. On the right panel, mean small-worldness is plotted as a function of the group (ELGA vs late-preterm) at the selected threshold. Error bars indicate one standard error of the mean.

Figure 2. Panel a: An example of normalized coherence graphs (averaged across subjects for each group) obtained after pruning the individual adjacency matrices at a threshold of 0.5 in the theta band. The color code correspond to the mean coherence value distribution within each group (from 0 – no connection – to the maximum reached value for the group). Panel b: difference between the local strength parameter (i.e., one value for each channel) of the ELGA and the late-preterm groups, in theta frequency band, plotted on the scalp. The bar indicates increased strength parameter in the right hemisphere of the late-preterm group with respect to ELGA infants. Panel c: mean strength parameter plotted as a function of the group (ELGA vs late-preterm) and the hemisphere (Left Hemisphere - LH vs Right Hemisphere - RH). A significant effect of the group emerged only in the right hemisphere. Error bars indicate one standard error of the mean.

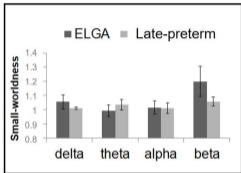
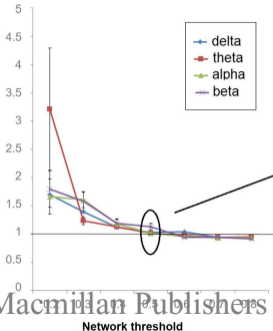
Table 1. Clinical data of the infants recruited for the study.

Clinical data	ELGA group (n=8)	Late preterm (n=8)
CA mean (range)	7 (5+3 – 8)	0+6(0+3 – 1)
PCA mean (range)	35 (34 – 35+1)	35 (34+3 – 36)
GA mean (range)	27 (23+2 – 27+6)	34+4 (34 – 35+2)
Birth Weight (gr)	974 \pm 234	2376 \pm 539
Birth Length (cm)	35 \pm 5	41 \pm 3
Birth CC	25 \pm 1	30 \pm 5
Male rate	4 (50%)	4 (50%)
1°-min Apgar score	7 \pm 1,27	8 \pm 0,75
5°-min Apgar score	8,25 \pm 0,83	9,2 \pm 0,95
Ph	7,27 \pm 0,09	7,25 \pm 0,06

GA: gestational age; CA: chronological age; PCA: postconceptional age (Engle, 2004); CC: cranial circumference;

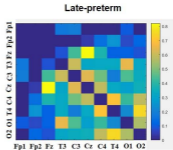
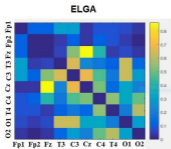
ELGA: extremely low gestational age.

Small-worldness

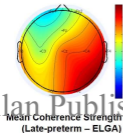


© Macmillan Publishers Limited, part of Springer

a)



b)



c)

