The Suppression Curve as a quantitative approach for measuring brain maturation in preterm infants

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HIGHLIGHTS

We apply the Suppression Curve for automated detection of maturational changes in multichannel preterm EEG.

The Suppression Curve reflects the decrease in EEG discontinuity with advancing postmenstrual age in healthy preterm infants.

Interburst Intervals values are calculated automatically and correlated with postmenstrual age.

ABSTRACT

Objectives: We apply the suppression curve (SC) as an automated approach to describe the maturational change in EEG discontinuity in preterm infants. This method allows to define normative values of interburst intervals (IBIs) at different postmenstrual ages (PMA).

Methods: Ninety-two multichannel EEG recordings from 25 preterm infants (born ≤ 32 weeks) with normal developmental outcome at 9 months, were first analysed using the Line Length method, an established method for burst detection. Subsequently, the SC was defined as the 'level of EEG discontinuity'. The mean and the standard deviation of the SC, as well as the IBIs from each recording were calculated and correlated with PMA.

Results: Over the course of development, there is a decrease in EEG discontinuity with a strong linear correlation between the mean SC and PMA till 34 weeks. From 30 weeks PMA, differences between discontinuous and continuous EEG become smaller, which is reflected by the decrease of the standard deviation of the SC. IBIs are found to have a significant correlation with PMA.

Conclusions: Automated detection of individual maturational changes in EEG discontinuity is possible with the SC. These changes include more continuous tracing, less amplitude differences and shorter suppression periods, reflecting development of the vigilance states.

Significance: The suppression curve facilitates automated assessment of EEG maturation. Clinical applicability is straight forward since values for IBIs according to PMA are generated automatically.

KEYWORDS

EEG

Preterm infant

Brain maturation

EEG discontinuity

Interburst intervals

Automatic

Line Length

Suppression Curve

1. Introduction

Despite cardiorespiratory advances in perinatal and neonatal intensive care, premature infants are still at high risk for neurological disabilities (Aarnoudse et al., 2009; Back and Miller, 2014; Larroque et al., 2008; Mwaniki et al., 2012). Early identification of at risk infants can improve short-term neuroprotective measures, and can ameliorate supportive measures in future life. Intensive brain monitoring in this vulnerable period of care, can help to identify the influence of various endogenous and exogenous disturbances on cortical activity and maturation (Chau et al., 2013; Kostovic and Jovanov-Milosevic, 2006; Kostovic and Judas, 2010; Victor et al., 2005b; Wikström et al., 2008).

Early cortical brain activity alternates between two activity modes. Periods of relative quiescence (interburst intervals) are interrupted by spontaneous activity transients (SATS or bursts) (Tolonen et al., 2007; Vanhatalo and Kaila, 2006). Bursts and interburst intervals (IBIs) will change with development and are influenced by medication as well as brain lesions (Hellström-Westas and Rosén, 2005; Iyer et al., 2015; Malk et al., 2014). Discontinuous activity (tracé discontinue) is the characteristic EEG pattern below 30 weeks postmenstrual age. Gradually, the electroencephalographic patterns evolve into a more continuous pattern (tracé continue) as a marker of maturation towards term age (André et al., 2010; Hayakawa et al., 2001; Scher, 1996; Vecchierini et al., 2003). Abnormal discontinuity for age (also referred as IBI duration) is the most studied feature in the preterm infant, associated with cerebral pathology, immaturity and adverse neurological outcome (Biagioni et al., 1994; Conde et al. 2005; Holmes and Lombroso et al., 1993; Menache et al., 2002; Wikström et al., 2012). However, accurate outcome prediction based on one single parameter may not be robust enough due to both physiological and technical shortcomings, and therefore, additional objective maturational parameters would fill the gap. Brain function can be transiently or permanently disturbed by various factors. To understand better physiological and pathological maturational processes in the preterm brain, continuous, consecutive and multichannel EEG measurements are essential. However, visual EEG interpretation requires expertise, and there are no standardized classification methods. A quantitative analysis of EEG activity, including automated analysis of selected maturational features of cortical function, would create a more objective and accurate classification scheme for preterm EEG.

Different burst detection algorithms for multichannel EEG are described in the literature (Jennekens et al., 2011; Koolen et al., 2014a; Murphy et al., 2015; Palmu et al., 2010). These studies were intended to improve algorithm performance and compared with interobserver agreement. Based on these automated algorithms, a possibility has been opened to study the developmental changes in the level of EEG discontinuity, and this without human intervention. Niemmarkt et al. (2010) tested the maturational trend with an interburst/burst ratio algorithm in 18 healthy preterms, however this study used only one bipolar EEG signal (C3-C4). No further studies have been performed on a larger dataset with consecutive, multichannel EEG recordings to verify the performance of any of these algorithms to assess global maturational changes in the discontinuous EEG pattern.

We apply a method, which quantitatively describes the maturational changes in EEG discontinuity in preterm infants with normal neurodevelopmental outcome at the corrected age of 9 months. This quantitative metric, the suppression curve, uses a single feature, the Line Length (LL). It has been shown in previous work to accurately detect high activity epochs (burst) and suppressed parts (IBIs) in long EEG recordings (Koolen et al., 2014a). In the present study, we want to assess whether the global developmental changes in the level of discontinuity, detected by the suppression curve, are robust enough to use it as a maturational feature. Second, we will improve the clinical applicability by presenting age-specific values for interburst intervals.

2. Methods

2.1 Data Acquisition

The study was performed at the Neonatal Intensive Care Unit of the University Hospitals of Leuven, Belgium during the period from February 2013 to September 2014 and approved by the Ethics Committee of the University Hospitals of Leuven, Belgium. All preterm infants with gestational age (GA) ≤ 32 weeks were eligible to enter this EEG study. Preterm infants were consecutively enrolled in the study after informed parental consent. The main dataset consisted of 149 recordings in 41 infants (GA ≤ 32 weeks) and all included infants had at least 2 consecutive recordings to assess brain development. From this larger dataset, 25 premature infants were retrospectively selected based on strict inclusion criteria: 1. normal neurodevelopmental outcome score at 9 months corrected age (Bayley Scales of Infant Development-II, for mental and motor function > 85), 2. no use of any sedative or anti-epileptic medication during EEG registration and 3. the absence of a severe cerebral lesion (normal cerebral ultrasonography or intraventricular hemorrhage (IVH) grade $\leq$ II, no periventricular leukomalacia or ventricular dilatation $> p97$).

2.2 EEG monitoring

The first EEG measurements were obtained as soon as the infant was clinically stable, between the first and the third week of life and then one EEG recording every 2 weeks up to transfer or discharge at home. These time points are a compromise between the accuracy of an identification of developmental features and the minimization of drop outs of the very preterm and critical sick newborns in the initial postnatal period. All infants underwent at least two and a maximum of six EEG recordings, depending on their stay at our NICU, resulting in 92 recordings, ranging from postmenstrual age (PMA, gestational age + postnatal age) 27 weeks to 42 weeks.

The optimal recording time we aimed for was 4 hours to record multiple vigilance states: mean 381 minutes (median (IQR) 254 min (219-316) min, minimum 115 min, maximum 1558 min). Feeding and care were carried out according to normal routine of the NICU, preferentially before and after the EEG measurement. Kangaroo Care was allowed and encouraged during the recordings as part of the application of the Newborn Individualized Developmental Care and Assessment Program (NIDCAP). Occasionally, in some infants, minimal duration of EEG recording was reduced due to clinical contingencies, but a complete sleep cycle was always acquired, in according to neonatal EEG surveillance guidelines (André et al., 2010).

All EEG measurements were recorded at 250 Hz, with a filter bandwidth of 0.27 Hz-70 Hz and 8 electrodes (Fp1, Fp2, C3, C4, T3, T4, O1, O2) placed according to the 10-20 standard locations and reference electrode Cz (BRAIN RT, OSG equipment, Mechelen, Belgium). Polygraphic monitoring included a channel for respiratory activity, electrocardiogram and oxygen saturation.

2.3 Automated analyses

Preprocessing the data involved applying a 50 Hz Notch filter and a 1-20 Hz band pass filter to capture the interesting burst information present in this frequency range, no additional artefact correction was performed on these recordings. Multichannel EEG recordings in unipolar mode were further analysed using the Line Length method, which includes th.4 characteristics of both amplitude and frequency content, and serves for automatic burst detection (Koolen et al., 2014a). In the first part of the algorithm, each EEG channel is divided into consecutive segments of 1 second (with an overlap of 0.12 second). Next, the Line Length value is calculated for each 1 second-segment and then normalized by dividing through the total line length of a 150 seconds- window. Normalization is necessary to average

out the effect of amplitude differences between EEG channels due to different electrode placement. After normalization, the median value over all 8 EEG channels is obtained for each segment: L_n .

In the second part of the algorithm, the suppression curve (SC) is derived, which represents the level of discontinuity in the EEG (different EEG states: discontinuous or continuous EEG). Each sample of the SC is calculated as the difference between the mean and the median of the median curve (L_n). In this way, the level of discontinuity is described for every EEG segment of 150 seconds, resulting in the suppression curve over time (derived from a multiple-hour recording). EEG parts with long IBI lengths resulted in high suppression values (larger difference between mean and median), whereas continuous EEG with short or even no IBIs resulted in lower suppression values. In this case, the mean and median of the L_n have comparable values. This is shown in Fig. 1, in which the suppression curve is depicted for discontinuous and more continuous parts of the EEG recording.

Fig. 1

Based on the same metric Line Length, we developed an accurate burst and IBI detection algorithm (Koolen et al., 2014a). All interburst intervals are extracted from the full EEG recording.

2.4 Statistical analyses

For each individual patient, the mean of the suppression curve (means SC) was calculated for each recording obtained at different ages and correlated with the postmenstrual age. To test the true correlation between EEG measures and PMA in the presence of repeated measures, two components were considered: between- subject and within-subject correlations. Repeated Measurements ANOVA proc mixed models was used (Bland and Altman, 1995a, 1995b; Roy A., 2006). In order to analyse the different measurements at various time points, we defined 6 age groups according to PMA, covering two weeks difference in PMA (group 1: PMA <28 weeks PMA, group 2: 28-30 weeks PMA, group 3: 30-32 weeks PMA, group 4: 32-34 weeks PMA, group 5: 34-36 weeks PMA, group 6: > 36 weeks PMA). Boxplots were derived to show distribution patterns of the mean SC and the standard deviation (SD) of the SC between different PMA groups. An analysis of variance (ANOVA) was used to test whether changes in the mean or the SD of the mean SC were significant different between subsequent age groups.

The IBI 95th percentile was calculated for all recordings. This reflects the maximum IBI length, since the maximum IBI itself is too sensitive for outliers as it is just one single value (André et al., 2010; Victor et al., 2005a). Repeated Measurements ANOVA proc mixed models was used to test whether differences in IBI 95th percentile were significant and could be associated with PMA for both the intra-individual and inter-individual correlations. Boxplots and the median of the IBI 95th percentile were calculated for every age group. Significance was defined as $p < 0.05$. Statistical analyses were performed in MATLAB and SAS 9.4.

3. Results

Twenty-five infants (mean GA 28.7 weeks (24.6-32), birth weight 1277g (540g-2540g), male/female ratio 9:16) with normal neurodevelopmental follow-up at a corrected age of 9 months were selected for further analysis. There is a wide range of illness severity in the study population. The demographics of the cohort and clinical details of the data are summarized in Table 1 and Supp. Fig. 1.

There is a clear overall decrease in mean SC values of all infant's recordings over the course of early development, with a statistically significant effect for PMA on both intra -and inter-individual evolution

of the mean of the SC (RM ANOVA proc mixed models $p < 0.001$ each) (Fig. 2a and Supp. Fig. 2). The largest developmental change in EEG discontinuity is observed around 30 weeks (boxplots of the mean SC: median value for 28-30 weeks: 0.17, 30-32 weeks: 0.10 and 32-34 weeks: 0.05, ANOVA $p < 0.05$), (Fig. 2b). Mean SC value is below 0.25 even in the smallest age group (26-28 weeks PMA), from 34 weeks onwards, mean SC was below 0.05, reflecting the presence of more continuous EEG. As shown in Fig. 3, there is a strong linear correlation between the mean SC and PMA till 34 weeks.

Fig. 2

When maturing, a more cyclic pattern is seen in the SC, representing alternating continuous and discontinuous EEG (Fig. 3a-d). However, near term age, difference between discontinuous EEG and continuous EEG became smaller in the SC. This is represented in the decrease of the standard deviations of the SC as depicted in Fig 2c (all groups ANOVA $p < 0.001$). The largest differences were found around 32 weeks PMA and 36 weeks PMA ($p < 0.05$).

Fig. 3

The overall decrease in discontinuity is further reflected in the evolution of the interburst intervals. The IBI 95th percentiles decreased with advancing postmenstrual age, with a statistically significant effect of PMA on the intra-individual as well as the inter-individual evolution of the IBI 95th percentiles (RM ANOVA proc mixed models $p < 0.001$ each) (Fig. 4a). The median IBI 95th percentile group values are as follows: (<28 weeks PMA) 15.1s, (28-30 weeks PMA) 12.4s, (30-32 weeks PMA) 10.7s, (32-34 weeks PMA) 8.9s, (34-36 weeks PMA) 8.0s, (>36 weeks PMA) 6.5s (Fig. 4b).

*** Fig. 4***

Four EEG recordings had higher mean SC values as compared with the group results. These are the four outliers, respectively in group 30-32 weeks PMA, group 32-34 weeks PMA, group 34-36 weeks PMA, group > 36 week PMA, as depicted in Fig. 2b. These higher SC values are probably related to a shorter EEG recording time with relatively more discontinuous EEG (115 min to 180 min instead of 240 min).

4. Discussion

Assessment of consecutive multichannel EEG of preterm infants without major brain lesions who develop normally, is generally accepted as the closest approximation of normal brain maturation. (André et al., 2010; Hellström-Westas and Rosén, 2005; Niemarkt et al., 2010; Victor et al., 2005a) This study shows that automated analysis of the 'level of discontinuity', an important marker of brain maturation in preterm multichannel EEG, is feasible with the suppression curve. This method allows an objective observation of the evolution of EEG measures, which reflect brain development at an intra-individual level. Moreover, this study is clinically relevant as it adds automated, objective and normative values of interburst intervals at different postmenstrual ages, considered as a valuable prognostic feature in the literature (Holmes and Lombroso, 1993; Menache et al., 2002; Vecchierini et al., 2003; Wikström et al., 2012).

This work extends prior work as no other studies used automated analysis on multichannel EEG records to describe the maturational trend of EEG discontinuity. Niemarkt et al. (2010) assessed brain maturation based on automated analysis of a single channel. Although single channel EEG is easier to analyse, algorithm performance may not be a realistic estimate of its expected performance on multichannel EEG recordings (Jennekens et al., 2011; Koolen et al., 2014a; Palmu et al., 2010). Furthermore, quantitative definitions of burst and interburst intervals differ in the literature, however

IBIs in EEG are mostly defined as inactivity in more than one channel (André et al., 2010; Biagioni et al., 2007; Victor et al., 2005a). As proven by Kato et al. (2011), the evaluation of brain maturation was much more accurate on continuous, multichannel EEG than on amplitude-integrated EEG (aEEG) based on one or two channels.

This study demonstrates that the highest decrease in the suppression curve is before 30-32 weeks PMA, reflecting the overall discontinuous EEG activity typically observed in preterm ages of 24-30 weeks PMA (André et al., 2010; Scher, 1996; Vecchierini et al., 2003). As the preterm infant matures, differences between discontinuous EEG and continuous EEG become smaller, which is reflected by the decrease of the standard deviation of the SC. These observations include more continuous tracing, shorter suppressions (IBIs) in the discontinuous EEG periods, and less amplitude difference between different EEG periods. These early changes in EEG continuity reflect functional brain maturation with maturing of different vigilance states as sleep and wakefulness. Interburst intervals of respectively 8 and 6 seconds from 34 weeks onwards, together with less amplitude differences between continuous and discontinuous EEG at 36 weeks, reflect the transition to tracé alternant in quiet sleep. All these findings from the fully automated, objective suppression curve measures are in line with the visual interpretation of normal premature and neonatal EEG, associated with good neurodevelopmental outcome reported in literature (Holmes and Lombroso., 1993; Shellhaas et al., 2014; Scher et al., 2005; Wikström et al., 2012).

The strength of this method is its good performance on multiple hour recording and at different PMA. Murphy et al. (2015) did not provide a realistic estimate of how their method performs when generalizing over age or when using longer recordings. In addition, the present study is an extra validation of our algorithm presented in previous work in a different preterm cohort (Koolen et al., 2014a). Previous data set included infants with confounding conditions such as suspected seizures or intracranial haemorrhage, representing potentially more suppressed EEG patterns. For this study, we applied strict selection criteria, resulting in a small cohort. The lack of statistical significance between two PMA groups may be related to the smaller size of the groups when divided by age in this way. To more generalise these results, further research with larger sample size will be performed. However, this study population is still clinically representative due to a wide range of GA and illness severity in the cohort. Another major advantage of this method is that the technical applicability of this method is straightforward since no preprocessing or artefact removal is applied on these signals, such as published by De Vos et al. (2001). This may facilitate further work to implement the algorithm on larger datasets in clinical NICU settings.

There are some technical and methodological considerations that may limit the accuracy of this study. First, two technical considerations: EEG recordings containing repetitive artefacts will result in lower SC values as expected for age. The SC allows more global assessment by integrating over longer time periods, however it relies on continuous temporal behavior, such as vigilance state cyclicity, which is another challenge to handle (Iyer et al., 2015). Fluctuations of brain state can alter mean SC value. We found higher mean SC values in 4 EEG recordings which had shorter EEG recording time due to measurement conditions. In these infants, EEG records were ended ahead of time, with an interrupted sleep wake cycle and relatively more discontinuous EEG (corresponding to quiet sleep). Therefore, EEG epochs should be long enough and of the same duration to overcome technical artefacts and subtle differences in vigilance state. Hence, one can speculate that consecutive measurements will overcome these shortcomings as the global maturational trend of intra-patient development is more important and underscores the strength of this EEG measure. Second, we correlated mean SC and IBI 95th percentiles only with PMA and not with GA or other confounding factors (e.g. intrauterine growth restriction) (Niemarkt et al., 2010; Yerushalmy-Feler et al., 2014), which can influence more inter-individual correlations. Preterm EEG patterns may change as a function of gestational age and postmenstrual age. Changes in EEG discontinuity are most pronounced before 34 weeks, as shown in the results. As a consequence, there may be some variability in mean SC on a certain PMA due to GA.

However, electro-cortical and anatomical maturation in ‘healthy’ preterm infants can be estimated within ± 2 to 3 weeks PMA (André et al., 2010; Biagioni et al., 2007; Kato et al., 2011). We assume to find no deviations more than two weeks due to GA, since all infants under study had normal developmental outcome. Furthermore, EEG measurements were performed after the first 72h. Recent reports show that EEG dynamics changes substantially over the first 72h after birth (Schumacher et al., 2014; Victor et al., 2005b, West et al., 2006). In this study, EEG measurements were performed after the first 72h in order to have stable brain dynamics at the respective age groups.

Although most prior studies have focused on predicting long-term neurodevelopment based on measuring different features of brain activity during the first 72h of life, other studies have correlated EEG maturation with neurodevelopmental outcome (Hayashi-Kurhashi et al., 2012; Le Bihannic et al., 2012). However, there is still no consensus in the literature which age is the correct moment to assess ‘abnormal brain maturation’. The main interest of our study is pointed out by the individual maturational trend which can be observed with the suppression curve in a particular patient. As early brain activity relates to subsequent brain growth (Benders et al., 2015), differences in preterm brain maturation, measured with mean SC slopes, may reflect altered brain organization. Further studies are warranted to test whether this is a potential feature for predicting neurodevelopmental outcome with longer follow-up data. Potential value of this method is its usefulness in EEG which represent various changes in infant alertness. Further work may include to test how endogenous (brain lesions, intrauterine growth restriction) and exogenous (medication, kangaroo care) factors may influence the suppression curve.

To conclude, this method has potential for fully automated analysis of complex and time consuming EEG of preterm infants and can reproduce quantitative results in a new dataset. Promising maturational features (IBI and burst characteristics, interhemispheric synchrony (Koolen, Dereymaeker et al., 2014b, Koolen et al. 2016) are extracted from the most discontinuous periods. Hence, detection of these discontinuous periods by using the suppression curve opens the way for further automated assessment of EEG maturation. Automated detection of normative values of interburst intervals can facilitate clinical practice.

CONFLICT OF INTEREST STATEMENT: None of the authors have potential conflicts of interest to be disclosed

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Table 1: Demographic and clinical data of the study population**N=25**

Gestational age (weeks)	28.7 ± 2.3 (range: 24.6 -32)
Male:Female	9:16 (36%:64%)
Birth weight, g	1277 ± 460 (range: 540-2540)
Respiratory support	
Conventional mechanical ventilation (SIMV)	9 (range: 1 day - 28 days)
High-frequency oscillation ventilation	3 (range: 8 days - 13 days)
Nasal continuous positive airway pressure	24 (range: 1 day - 63 days)
Patent Ductus Arteriosus	
Drug (Ibuprofen)	6 (24%)
Surgical ligation	2 (8%)
Postnatal Corticosteroids (Hydrocortisone)	3 (12%)
Sepsis/ Antibiotics > 72h	15 (60%)
Necrotizing Enterocolitis (NEC)	2 (8%)
Cranial Ultrasound	
Normal	15 (60%)
Grade I Intraventricular haemorrhage (IVH)	3 (12%)
Grade II Intraventricular haemorrhage (IVH)	2 (8%)
Flaring < 14d	5 (20%)
Retinopathy of prematurity (ROP) with laser therapy	3 (12%)
Bronchopulmonary dysplasia (BPD)	2 (8%)
Developmental outcome at corrected age of 9 months	
Bayley Mental - II (MDI)	109 ± 12
Bayley Motor - II (PDI)	113 ± 15

Values are expressed as n (%), mean ± SD, range. BPD: need for supplemental oxygen/ventilation at 36 weeks PMA. Sepsis: positive blood culture or highly suspected with antibiotic treatment > 72h

Figure Legends

Fig. 1: The suppression curve derived from a multiple-hour recording. The suppression curve is depicted for discontinuous (black) and more continuous (gray) parts of the EEG recording.

Fig. 2: (A) The overall decrease in mean SC values of 92 multichannel EEG recordings over the course of early development (scatter plot for 25 preterm infants). There is a statistically significant effect for PMA on both intra-individual and inter-individual evolution of the mean of the SC ($p < 0.001$ each). We defined 6 age groups according to postmenstrual age (PMA), covering two weeks difference in PMA age (red: PMA <28 weeks PMA, black: 28-30 weeks PMA, blue: 30-32 weeks PMA, green: 32-34 weeks PMA, pink: 34-36 weeks PMA, yellow: > 36 weeks PMA). (B) Boxplots representing the developmental changes of the mean SC over the different PMA groups. A significant developmental change in EEG discontinuity is observed around 30 weeks and marked with asterisk (ANOVA $p < 0.05$). (C) Boxplots representing the developmental changes of the standard deviation (SD) of the SC over the different PMA groups. Near term age, difference between discontinuous EEG and continuous EEG became smaller in the SC. Significant differences were found around 32 weeks PMA and 36 weeks PMA and marked with asterisk (ANOVA $p < 0.05$).

Fig. 3: The decrease in mean SC value over the course of early development (scatter plot for 25 preterm infants). There is a strong linear correlation between the mean SC and PMA till 34 weeks. The suppression curve from 4 hours multichannel EEG recording at different PMA in one patient. (A) High suppression values represent mainly discontinuous EEG. (B-C) When maturing, a more cyclic pattern is seen in the SC, representing alternating continuous and discontinuous EEG. (D) At term age, difference between discontinuous EEG and continuous EEG became smaller in the SC.

Fig. 4: The IBI 95th percentiles of 92 multichannel EEG recordings, performed in 25 preterm infants. (A) The IBI 95th percentiles decreased with advancing postmenstrual age, with a statistically significant effect of PMA on the intra-individual as well as the inter-individual evolution of the IBI 95th percentiles ($p < 0.001$ each). (B) Boxplots representing the 95th percentile over the different PMA groups. The median IBI 95th percentile group values are as follows: (<28 weeks PMA) 15.1s, (28-30 weeks PMA) 12.4s, (30-32 weeks PMA) 10.7s, (32-34 weeks PMA) 8.9s, (34-36 weeks PMA) 8.0s, (>36 weeks PMA) 6.5s.

Supp. Fig. 1: Distribution of preterm neonates across gestational age.

Supp. Fig. 2: The intra-individual evolution of the mean SC across postmenstrual age, plotted for all patients separately. Both the intra-individual and the inter-individual correlations are significantly negative ($p < 0.001$ each) and reflect the developmental evolution of this EEG measure.