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Early Brain Activity Relates to Subsequent Brain Growth in Premature Infants

Manon J. Benders[1,2], Kirsi Palmu[3,4], Caroline Menache[1], Cristina Borradori-Tolsa[1], Francois Lazeyras[5], Stephane Sizonenko[1], Jessica Dubois[1,6], Sampsa Vanhatalo[4,†] and Petra S. Hüppi[1]

[1]Division of Development and Growth, Department of Pediatrics, Children's Hospital, University of Geneva, Geneva, Switzerland, [2]Department of Neonatology, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands, [3]Department of Biomedical Engineering and Computational Science, School of Science, Aalto University, Helsinki FIN-00076, Finland, [4]Department of Children's Clinical Neurophysiology, Children's Hospital, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland, [5]Center for Biomedical Imaging (CIBM), Department of Radiology, University Hospital of Geneva, Geneva, Switzerland and [6]Cognitive Neuroimaging Unit U992, NeuroSpin, INSERM-CEA, Gif-sur-Yvette, France

[†]Sampsa Vanhatalo shared last authorship, equal contribution.

Address correspondence to Manon Benders, Department of Neonatology, Wilhelmina Children's Hospital, E 04.123.1, PO Box 85090, 3508 AB Utrecht, The Netherlands. Email: m.benders@umcutrecht.nl

Recent experimental studies have shown that early brain activity is crucial for neuronal survival and the development of brain networks; however, it has been challenging to assess its role in the developing human brain. We employed serial quantitative magnetic resonance imaging to measure the rate of growth in circumscribed brain tissues from preterm to term age, and compared it with measures of electroencephalographic (EEG) activity during the first postnatal days by 2 different methods. EEG metrics of functional activity were computed: EEG signal peak-to-peak amplitude and the occurrence of developmentally important spontaneous activity transients (SATs). We found that an increased brain activity in the first postnatal days correlates with a faster growth of brain structures during subsequent months until term age. Total brain volume, and in particular subcortical gray matter volume, grew faster in babies with less cortical electrical quiescence and with more SAT events. The present findings are compatible with the idea that (1) early cortical network activity is important for brain growth, and that (2) objective measures may be devised to follow early human brain activity in a biologically reasoned way in future research as well as during intensive care treatment.

Keywords: cortical folding, EEG, MRI, preterm infants, volumetric measurements

Introduction

Improved treatment in the neonatal intensive care unit (NICU) has led to an increased survival of preterm infants; however, the rate of poor neurodevelopmental outcome has remained (Costeloe et al. 2012). This has shifted the attention from mere survival to optimizing brain protection during the vulnerable perinatal period. Consequently, there has been an increasing demand to monitor brain function during the critical early days and weeks (Klebermass et al. 2011; Griesmaier et al. 2012) after preterm birth.

Electroencephalography (EEG) is a readily available tool that can be used for both basic research and a long-term monitoring of brain function in the NICU. Its real-time (bedside) interpretation is widely based on its time-compressed representation called amplitude-integrated EEG (aEEG; a.k.a. cerebral function monitor, CFM). Visual pattern recognition may distinguish aEEG trend patterns, such as sleep–wake cycling, seizures, and burst suppression (Burdjalov et al. 2003; Hellström-Westas et al. 2006). Such visual assessment of aEEG trends has proved to be useful in a range of NICU situations, including early assessment of brain injury in preterm infants (Sisman et al. 2005; Hellström-Westas et al. 2006; Klebermass et al. 2006; Niemarkt, Andriessen, Peters, Pasman, Blanco, et al. 2010; Niemarkt, Andriessen, Peters, Pasman, Zimmermann, et al. 2010). The preterm brain is known to be most vulnerable during the first postnatal days, and the main challenge in the NICU care is to maintain brain structural and functional integrity.

In this context, it is conceivable that recent interest has focused on assessing brain activity of early preterm babies as a biomarker that could be used to predict their future neurological development. Some studies using relatively rough EEG-based measures have already shown that early brain activity is associated with later neurological outcome. This association is independent of brain injury, and the clearest relationship has been shown between poor background patterns and poor neurodevelopmental outcome (Wikström et al. 2012; Olischar et al. 2013).

Recent basic neuroscience studies in vitro and in vivo on various parts of brain and spinal cord have shown that the early electrical activity in the neuronal networks is fundamentally important for successful brain development by providing trophic support for neuronal survival, as well as endogenous guidance for activity-dependent wiring (Khazipov and Luhmann 2006; Hanganu-Opatz 2010; Kilb et al. 2011; Tolner et al. 2012) during a developmental time period that equals to the last few months of pregnancy in humans (Kostović and Judaš 2010). Further advance in this field is likely to come from a merge of basic neuro-scientific knowledge and studies on live human babies that are only available in the neonatal intensive care environment.

Recent advances in the digital signal analysis of neonatal EEG and magnetic resonance imaging (MRI) have opened ways for an objective quantitation of early brain activity (Palmu, Stevenson et al. 2010; Palmu, Wikström, et al. 2010; Jennekens et al. 2012; O'Reilly et al. 2012; Palmu et al. 2013) and structural brain growth (Hüppi et al. 1998; Dubois, Benders, Borradori-Tolsa, et al. 2008; Dubois, Benders, Cachia, et al. 2008; Dubois et al. 2010; Kapellou et al. 2011; Rathbone et al. 2011), respectively. Two distinct strategies have been developed for an objective analysis of the preterm EEG. First, it is possible to quantify amplitude content of the EEG over time by subdividing it into EEG signal peak-to-peak amplitude (ppA) categories, hereafter called band A–E (O'Reilly et al. 2012). Secondly, recent neurobiological work has revealed distinct brain events, spontaneous activity transients (SATs; a.k.a. bursts; Vanhatalo et al. 2005;

Vanhatalo and Kaila 2006) in the preterm EEG, and these events can be automatically detected from long-term EEG recordings of preterm infants (Palmu, Stevenson, et al. 2010; Palmu, Wikström, et al. 2010; Jennekens et al. 2012; Palmu et al. 2013).

The present study was set out to assess (1) whether early brain activity during the first postnatal days in preterm infants is related to the structural maturity of the brain measured on early MRI, and moreover, (2) whether level of this early brain activity correlates with the subsequent rate of brain growth during the first months of postnatal life of preterm born babies until they reach term equivalent age.

Patients and Methods

Patients

This study was part of a larger preterm cohort study "Monibrain" approved by the local ethical review board and performed at the Children's Hospital in Geneva, with full written informed consent of parents. Only preterm infants with 2 good quality sequential MRI assessments at birth and at term equivalent age, and reliable EEG tracings were included in this study. Furthermore, only infants without severe brain lesions were included [punctate white matter lesions (PWMLs) and intraventricular hemorrhage less than grade 3], because it was impossible to perform reliable volumetric brain segmentation on the severely damaged brain. Twenty-one preterm newborns [mean gestational age (GA) at birth was 29.3 ± 2.5 weeks] were included for this study. A total of 58 were initially identified at GA below 36 weeks, thereafter 7 died or did not have a second MRI, 16 had unreliable tracing, 9 movements artifacts on MRI, 3 severe brain lesions, and 2 were excluded because of intrauterine growth restriction (birth weight <2 SD) known to affect brain growth. Table 1 presents the patients details.

Four infants received morphine/midazolam as sedation on mechanical ventilation. Blood gases were sampled every 4–6 h during the period of EEG recordings in the first few days of life; all infants were stable and did not have extreme (higher or lower) levels in CO_2 that could confound the assessment of EEG activity (Victor et al. 2005; Wikström et al. 2011).

EEG Acquisition

The 2-channel BRM3 brain monitor (Natus, USA; former Brainz monitor) was used for collecting EEG signals during the first 72 h after birth. This EEG system stores data at 256 Hz, and the signal is filtered using a combination of first-order high-pass filter at 1 Hz and fourth-order low-pass Butterworth filter at 50 Hz. Hydrogel electrodes (Hydrospot, Physiometrix, Inc., North Billerica, MA, USA) were placed bilaterally on the scalp at central and parietal positions (C3, C4, P3, and P4) according to the international 10–20 system.

Table 1
Patients' characteristics

Gestational age (weeks)	29.3 ± 2.5 range: 25.6–35.6
Birth weight (kg)	1.242 ± 0.469 range: 770–2730
Lesions (PWML) (*n*)	5
Sedation (*n*)	4
Gender (*n*)	10 girls
GA at MRI1 (weeks)	30.5 ± 2.4 range: 26.7–35.7
GA at MRI2 (weeks)	40.5 ± 0.8 range: 39.0–42.9

Values are denoted as mean ± SD or *n*.

EEG Analysis

All EEG were first examined visually using the clinical BrainZ Analyzer software to identify possible artifacts, as well as to exclude severe abnormalities from the further analysis. In addition to visual identification, some artifacts were also automatically identified and thereby excluded by the clinical software (shown with a colored tint in the review display, when impedance was too high). The EEG system has a default of generating 3 bipolar derivations (C3–P3, C4–P4 and P3–P4) from the recorded signals. However, after thorough evaluation of all EEG signals, we decided to use only the cross-cerebral derivation (P3–P4; a.k.a. bi-parietal signal) to minimize uncertainties related to mere technical and/or physical factors, such as possible asymmetries due to subcutaneous swelling that is known to affect EEG amplitudes.

For the quantitative EEG analysis, we employed 2 distinct, conceptually independent paradigms. First, we used the recently established "amplitude-based EEG measures" tailored for neonatal data (O'Reilly et al. 2012), which are based on estimation of wave amplitudes irrespective of underlying brain phenomena. Secondly, we used "event-based EEG measures" that were recently optimized in our laboratory (Palmu, Stevenson et al. 2010; Palmu, Wikström, et al. 2010) to quantify the occurrence of brain events (SATs; Vanhatalo et al. 2005); for more details about nomenclature, see Table 1 in Vanhatalo and Kaila (2006) that are known to be crucial for brain maturation (Hanganu-Opatz 2010; Kilb et al. 2011). By using this combination of measures, we aimed to find synergies from the as yet distinct, parallel streams of neonatal EEG analysis, and to be able to find parameters that could be potentially applied as early functional biomarkers in future scientific studies, or as putative indices in the prospective neonatal EEG monitoring. The rationale of these paradigms is schematically visualized in Figure 1 and further described below.

Amplitude-Based EEG Measures

We selected representative 1 h long epochs of EEG between 20 and 24 h after birth (mean 22.1 ± 0.4 h), since single-channel aEEG /EEG contains predictive information on long-term outcome in very preterm infants at 24 h of age (Wikström et al. 2012). The amplitude analysis was performed using the customized software Chart Analyzer (Brainz Instruments). This software has an in-built analytic paradigm for estimating ppAs at 2 s time intervals as recently described (O'Reilly et al. 2012). This paradigm has been named range EEG (rEEG), and it was developed as a putative substitute for the aEEG paradigm. The main advantages of rEEG are its notably lower sensitivity to noise, its direct correspondence to the amplitude of the original (raw) EEG signals, and unlike the aEEG/CFM paradigm, it is less biased by the *a priori* choice of EEG frequencies (O'Reilly et al. 2012).

Amplitude Bands and Their Reasoning

In the rEEG paradigm, the measured ppA values are classified into 5 different amplitude categories designated as bands A–E (see also Fig. 2*B*): band A 0–10 μV, band B 10–25 μV, band C 25–50 μV, band D 50–100 μV, and band E >100 μV. The quantitative output of rEEG in our study was the percentage of time represented by each amplitude category over the 1 h epoch.

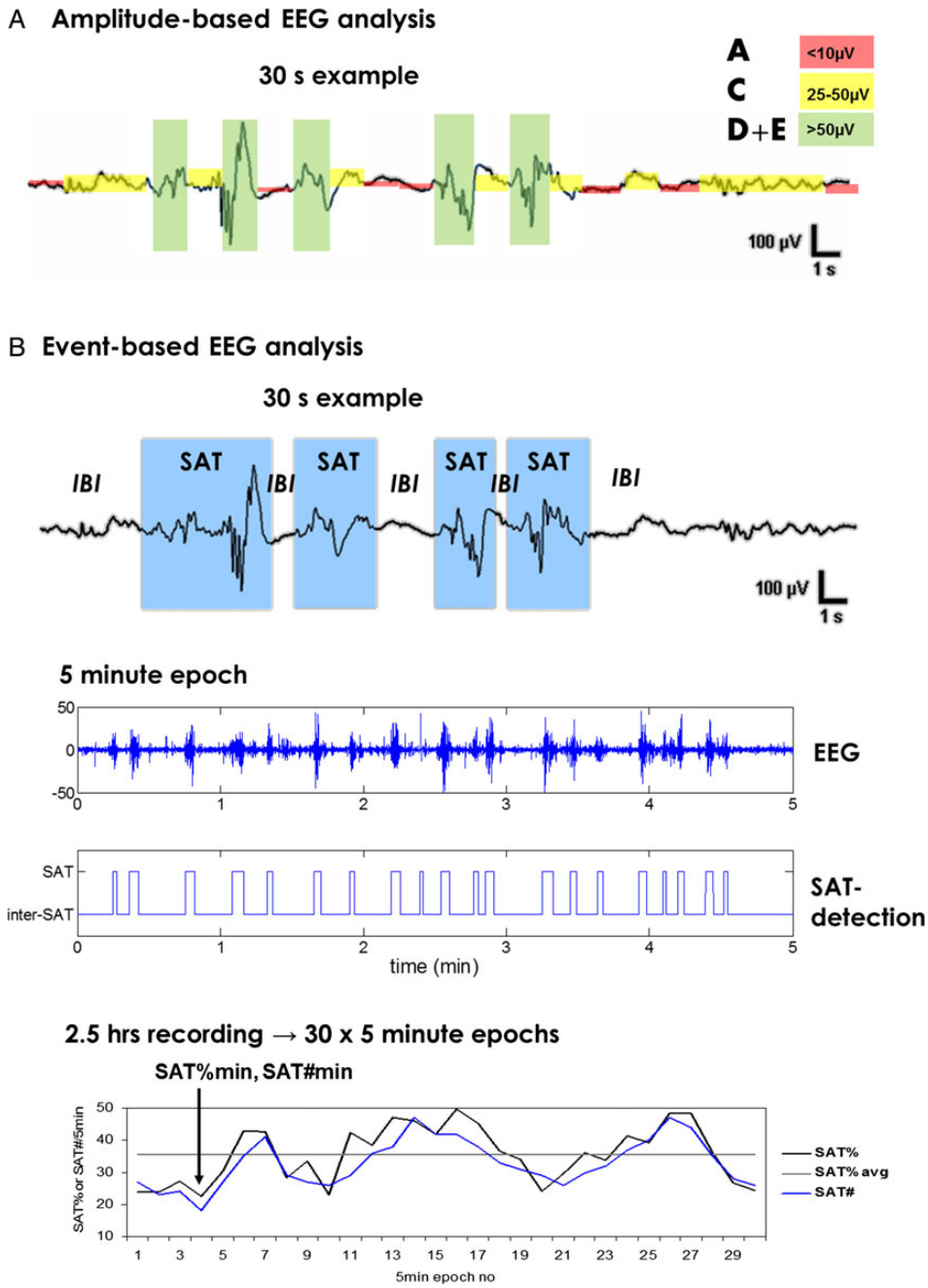

Figure 1. Schematic presentation of the 2 EEG analysis paradigms. (*A*) The amplitude-based EEG analysis based on ppAs where amplitude categories are customized (*A*, *C*, *D* + *E*) for neonatal data (O'Reilly et al. 2012). The output of this analysis presents the proportion of time covered by each amplitude category, and the estimation of wave amplitudes is mathematical. (*B*) The event-based EEG analysis begins from detection of brain events called SAT (Vanhatalo et al. 2005; Vanhatalo and Kaila 2006), and the intervals between these detections are called inter-SAT (Tolonen et al. 2007) or IBIs. The detections are performed on a continuous basis, but the metrics are subsequently computed for 5 min epochs as displayed in the lowest graph.

The amplitude bands are chosen to reflect distinct levels of background activity. The lowest amplitude, band A, reflects an almost flat EEG signal. The highest bands, band D and E, were combined together (band DE). Band DE reflects most of the physiological and pathophysiological bursts with highest amplitudes (voltage above 50 μV). It is hence conceivable that the rEEG bands are also reflected in the well-known quantitative and qualitative maturation of brain activity (André et al. 2010; O'Reilly et al. 2012).

In this context, it is important to note that the term (amplitude) "band" is not related to the common concept of EEG "frequency bands." The term "band" to describe different amplitude categories was originally motivated by the possibility to display them as temporally compressed, colored bands in the monitoring software akin to the classical density spectral array in many medical applications.

Event-Based EEG Measures

Using the BrainZ Analyzer reading software, we selected representative continuous 2.5 h long segments (mean age at the epoch onset 29 ± 11 h). The selected segments were then imported into Matlab environment, where SAT events were

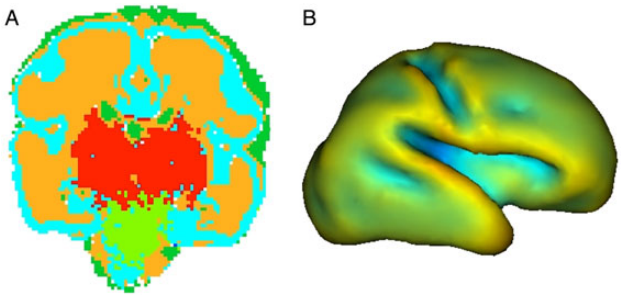

Figure 2. Image postprocessing example of a premature newborns of 28 weeks GA, showing tissue classification (Hüppi et al. 1998) resulting from T_2 and T_1 images (blue: developing cortex; red: BGTh; orange: developing white matter; light green: myelinated white matter and green: cerebrospinal fluid). (*B*) Inner cortical surface reconstruction of the same patient: example of the 3D interface between the developing cortex and white matter. The colors outline the surface curvature (Dubois, Benders, Cachia, et al. 2008).

automatically detected using an NLEO-based algorithm (automated burst detector that uses a nonlinear energy operator; see Fig. 2*B*; Palmu, Stevenson, et al. 2010). This algorithm was recently validated and optimized (Palmu, Stevenson, et al. 2010; Palmu, Wikström, et al. 2010) for the early preterm EEG signal collected with another EEG system (NicOne, Cardinal Healthcare/Natus, USA). The amplifier-dependent filtering characteristics may modestly affect the absolute values seen in our detection (Palmu et al. 2013); however, there is no reason to expect that it could confound analyses of datasets where all EEG was collected using the same amplifier (here, BRM3, BrainZ). This algorithm outputs a binary classification of each EEG sample (256 samples per second) as either SAT or inter-SAT (a.k.a. interburst interval, IBI; Wikström et al. 2008, 2012), which can then be used for computing event-based metrics. In the present study, we chose to construct 3 different metrics that were designed to capture distinct aspects of the brain activity.

Event-Based Metrics and Their Reasoning

We first measured the degree of inactivity by defining the longest quiescence between consecutive SAT events, called "maximum IBI" (IBImax). This measure is conventional in the neonatal literature (cf. Hahn et al. 1989), and it is taken as a single value from the longest IBI in the given EEG segment (here 2.5 h). We then assessed the amount of brain activity by computing the minimum number of SAT events per minute (SAT#min) in successive 5-min epochs, which comes close to the previously reported frequency of spikes in the aEEG trend (called "bursts" in Wikström et al. 2008, 2012). Our prior studies have indicated that counting the events, whether SAT# or IBImax, may be unpredictably affected by the choice of detection thresholds and signal filtering, whereas estimating temporal percentage of detections is likely more robust to technical uncertainties (Palmu, Wikström, et al. 2010). Therefore, we complemented our analysis by also computing the percentage of time that was detected as SAT events (SAT%), taking the lowest 5-min epoch value in our analyzed 2.5 h recordings (SAT%min), or taking the average over the whole 2.5 h recording (SAT%avg). All these event-based metrics are obviously not fully independent. For instance, a long IBImax may often be found in babies with a lower SAT# or SAT%. The average SAT% (SAT%avg) over the whole 2.5 h epoch is presumably the most stable and comprehensive metric. It is important to note, however, that these detection-based metrics are neither mutually interchangeable nor directly complementary, and we will discuss below how their intrinsic behavior suggests them different potential for application into prospective research and clinical brain monitoring.

Magnetic Resonance Imaging Acquisition

Newborns underwent 2 MRI examinations. The study was conducted on a 1.5-T Philips Medical Systems (Intera or Achieva) or a 3-T Siemens Medical System (Avanto). The first MRI examination was performed as soon as possible after birth, when clinically stable, and the second at term equivalent age. They were fixed within a vacuum pillow, and special "mini-muffs" were applied on their ears to minimize noise exposure. Coronal images covering the whole brain were acquired using anatomical sequences (T_2-weighted fast spin echo sequence and 3D T_1-weighted fast gradient recovery sequence), with high spatial resolution ($0.7 \times 0.7 \times 1.5$ mm^3 or $0.8 \times 0.8 \times 1.2$ mm^3) as published earlier (Dubois, Benders, Cachia, et al. 2008; Dubois, Benders, Borradori-Tolsa, et al. 2008).

MRI Analysis

Data Postprocessing and Evaluation

The human newborn brain maturation was evaluated using image postprocessing tools that enabled the measurements of the cerebral tissue volumes on one hand (Hüppi et al. 1998), and quantification of the cortical gyri and sulci formation and surface on the other hand (Dubois, Benders, Cachia, et al. 2008; Dubois, Benders, Borradori-Tolsa, et al. 2008). White matter lesions described at early MRI were of limited spatial extent <2 mm, and <6, and sometimes localized in both cerebral hemispheres.

Brain Tissue Classification and Volumetric Quantification

First, for both the first and second MRI examinations, quantitative measurements of total cerebral brain volumes [TBVs, with the following tissue classes: cortical gray matter (CGM), basal ganglia/thalami (BGTh), nonmyelinated and myelinated white matter and cerebrospinal fluid] were performed using k-nearest-neighbor classification (Fig. 2*A*). This method is based both on the MRI signal intensity of the registered T_2- and T_1-weighted images (Hüppi et al. 1998; Inder et al. 2005) and on anatomic location to differentiate CGM and BGTh according to basal ganglia atlases for the preterm and term groups. TBV was considered to be the best marker to describe brain growth globally. Since white matter, also including the subplate, during the observed developmental period, and cortical gray matter are the major volumes of the brain, TBV seems to be a good expression of the 2 tissues undergoing the largest developmental changes during this period. BGTh volume distinct from CGM is reliably measured, in neonatal brain segmentation (Hüppi et al. 1998; http://neobrains12.isi.uu.nl). Its role in endogenous guidance for activity-dependent wiring of the cortex makes it an interesting anatomical entity to study (Kostović and Judaš 2007). Therefore, analysis was performed with TBV and volume of BGTh.

Cortical Surface and Sulcation Index Measurement

Furthermore, we applied an approach (Dubois, Benders, Borradori-Tolsa, et al. 2008; Dubois, Benders, Cachia, et al. 2008) to assess the degree of cortex gyrification on the inner cortical surface from T_2-weighted images of the first MRI

examination (Fig. 2*B*) (Dubois, Dehaene-Lambertz, et al. 2008) when gyrification is incomplete. The method is extensively described by Dubois, Benders, Cachia, et al. (2008 using the above-described segmentation method (Hüppi et al. 1998). To characterize the whole brain growth with age, the total closed surface of the cortex was calculated. The sulcation index characterized the proportion of sulci according to this closed surface. It has been shown in the previous study that these measurements of cortical morphology in the preterm newborn brain correlated highly with GA (Dubois, Benders, Cachia, et al. 2008).

Statistical Analysis

We will assess the relationship with the 2 different methods of EEG metrics between early brain activity and structural MRI measures shortly after birth, followed by the correlation between early brain activity and term equivalent age brain structures (BGTh and TBV). Furthermore, we will examine whether early brain activity relates to subsequent brain growth by showing a correlation to the rate of structural brain growth from preterm life to term equivalent age [(Volume at term MRI − Volume at early MRI)/(weeks in between)].

Due to the several week variance in the GA within our study group, we first estimated the relationship between EEG metrics and GA with the nonparametric Spearman correlation coefficient. As we found significant correlations, further analyses were carried out after controlling for GA using partial correlation analysis as implemented in SPSS. There were few babies with concurrent morphine medication ($n = 4$) or minor, punctate MRI lesions ($n = 5$), and hence we carried out primary analysis after controlling for these variables as well. A secondary analysis was subsequently done without controlling for medication and/or MRI lesions, because we also wanted to see if the relationship between early EEG metrics and brain growth could be seen in a more heterogeneous patient population. A significance level was set to $P < 0.05$, and we considered $P < 0.1$ as trend-level significance.

Results

Significant correlations were found between the GA and early MRI structural markers, in terms of cortical surface ($r = 0.91$, $P < 0.001$) and sulcation ($r = 0.95$, $P < 0.001$), and brain volumes (TBV $r = 0.93$, BGTh $r = 0.74$, $P < 0.001$) in agreement with previous studies (Hüppi et al. 1998; Dubois, Benders, Cachia, et al. 2008). Hence, all our further analyses were corrected for GA.

We will show the results for the 2 different EEG metric approaches in relation with (1) GA, (2) structural brain measures shortly after birth, and at term equivalent age, and (3) the rate of structural brain growth from preterm life to term equivalent.

Early EEG Metrics Versus GA

Amplitude-based EEG measures: No significant correlation was found between GA at birth and the amplitude measures band A and band DE, while a trend-level relationship was seen between GA and band C ($r = 0.69$, $P = 0.059$, Supplementary Fig. 1*A*).

Event-based EEG measures: As expected from the known maturation of the electric brain activity (Vanhatalo and Kaila 2006; André et al. 2010), some event-based EEG measures were found to be significantly correlated with the GA (IBImax: $r = -0.70$, $P < 0.01$; SAT%min $r = 0.554$, $P < 0.05$; Supplementary Fig. 1*B*; SAT#min $r = 0.617$, $P < 0.01$).

Early EEG Metrics Versus Structure in the Early Preterm Brain and Term Equivalent Age

Amplitude-based EEG measures: Normal EEG continuity, reflected in the band C, was found to correlate with the measures of cortical sulcation and surface early after birth (respectively, $r = 0.95$ and 0.94, $P < 0.001$, Supplementary Fig. 2*A*-1). The same measure of EEG continuity was also robustly correlated with TBV at early MRI ($r = 0.95$, $P < 0.001$). On the opposite, low background activity, expressed by band A, was correlated with smaller TBV ($r = 0.96$, $P < 0.001$) on early MRI. Increased activity (band DE) did not show a correlation with total brain volume early after birth.

At term equivalent age, there was no correlation with band C and brain structures. Suppressed brain activity, expressed by band A, however, showed a trend to lower TBV at term ($r = -0.80$, $P = 0.08$) (Supplementary Fig. 2*B*-1), and the increased activity (band DE) showed a significant correlation with higher TBV at term ($r = 0.83$, $P < 0.05$; Supplementary Fig. 2*B*-2).

Event-based EEG measures: We found that measures of early brain activity versus inactivity were differentially correlated with measures of cortical and subcortical structures. Longer periods of inactivity (higher IBImax) in the first day after birth were significantly correlated with less cortical surface ($r = -0.64$, $P = 0.01$) and lower volume of TBV ($r = -0.71$, $P < 0.01$) on early MRI. The volume of BGTh at term age was significantly larger in infants with higher SAT#min or SAT %min ($r = 0.73$, $P < 0.05$ and $r = 0.77$, $P < 0.01$, respectively).

Early EEG Metrics Versus Growth Rate During Preterm Life to Term Equivalent Age

Amplitude-based EEG measures: We found that early brain activity differentially correlated with the growth rates of brain structures. A higher level of brain activity, expressed by band DE, correlated with faster growth of TBV ($r = 0.85$, $P < 0.01$, Fig. 3*A*-1), and this was also strongly significant for BGTh volume growth ($r = 0.82$; $P < 0.05$, Fig. 3*A*-3). Low early cortical activity level, band A, showed an opposite trend in its correlation to the structural growth of total brain volume ($r = -0.458$, $P = 0.07$, Fig. 3*A*-2).

Event-based EEG measures: Higher occurrence of SAT events were strongly associated with faster growth of BGTh, as seen in all SAT-derived indices (SAT#min $r = 0.927$, $P < 0.001$; SAT%min $r = 0.922$, $P < 0.001$, Fig. 3*B*-3; SATavg $r = 0.882$, $P = 0.001$), whereas the average level of SAT events (SAT%avg) correlated at a trend level to the rate of growth of TBV ($r = 0.53$, $P = 0.07$, Fig. 3*B*-1). Shorter periods of inactivity (IBImax) were correlated with both a faster growth of TBV ($r = -0.678$, $P < 0.05$, Fig. 3*B*-2) and a more rapid growth of BGTh ($r = -0.667$, $P = 0.05$).

EEG metrics indices remained significant even when the correlation analysis was not controlled for morphine and/or MRI lesions, indicating a strikingly robust relationship between brain activity and growth.

Discussion

Our study on quantitative EEG metrics shows a significant positive correlation between early brain activity and brain growth, specifically BGTh growth, during human preterm life.

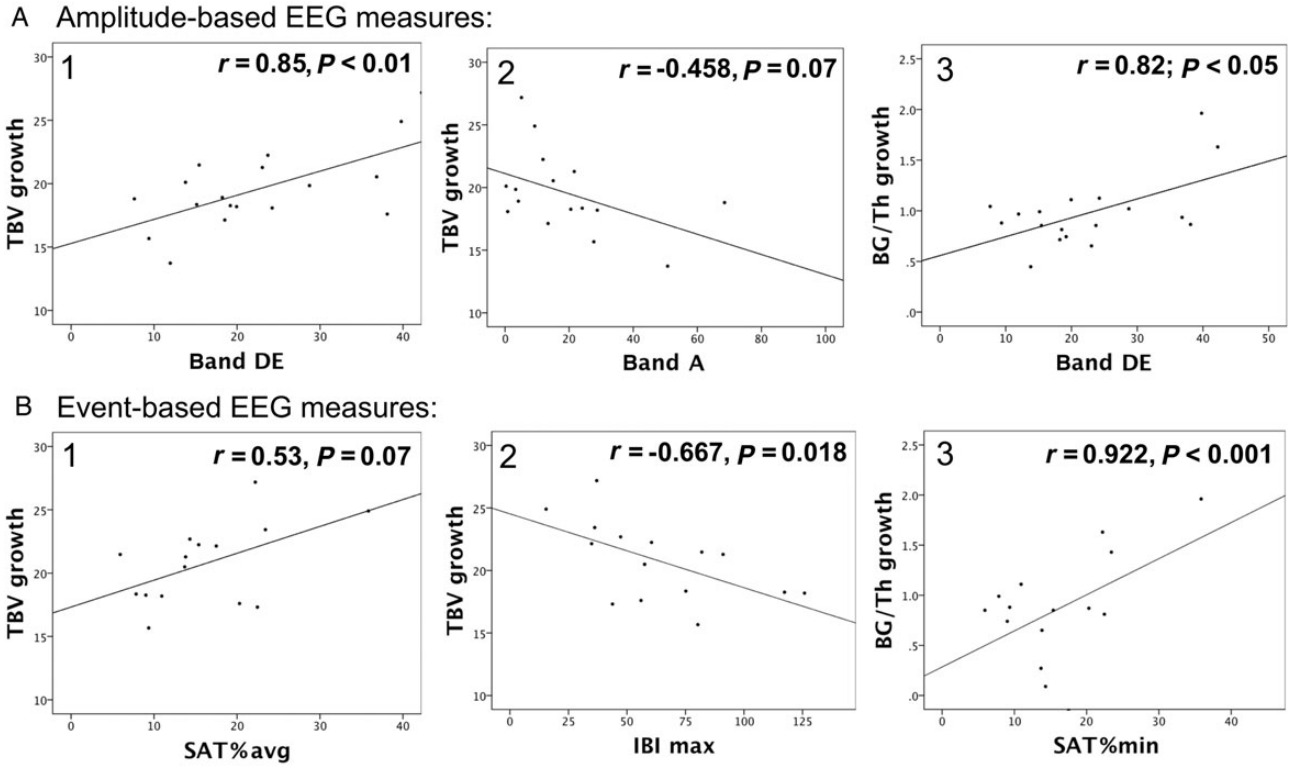

Figure 3. Correlation of early brain activity to the subsequent growth rate of brain structures. (*A*)Amplitude-based measures revealed a significant correlation between high EEG amplitudes (band DE; % of time per hour) and the growth of total brain volume (TBV (*A*1) as well as the BGTh growth (*A*3). Moreover, reduced level of early cortical activity, reflected in band A (% of time per hour), showed a negative trend with the rate of subsequent TBV growth (*A*2). (*B*)Event-based measures showed similar findings where SAT%, correlated at a trend level to the rate of growth of TBV (*B*1), significantly correlated with BGTh growth (*B*3). The reduced level of early cortical activity, interburst interval (IBImax), showed a significant negative correlation with the rate of subsequent TBV growth (*B*2), confirming the trend seen in *A*. Growth is expressed in mL/week.

Furthermore, we confirmed that early brain activity is related to GA and structural cortical morphology at birth illustrating structural and functional brain maturation. Previous studies have shown that EEG undergoes a profound developmental change during preterm life (André et al. 2010), and hence it expectedly correlates with measurable changes in the structural brain development (Biagioni et al. 2007), as was also shown in present work. However, our findings extend the earlier literature into 2 novel aspects. First, we implemented neurophysiologically reasoned and analytically robust, objective metrics of early brain activity. Secondly, we measured the developmental dynamics of structural growth by serial MRI, which readily overcomes the analytical challenges associated with studies that only assess the brain at single time point, mostly term equivalent age. Given the known heterogeneity in both EEG and MRI studies of human preterm babies, we found it striking that EEG metrics using 2 independent analytic approaches jointly support the notion that early brain activity relates to subsequent brain growth. Our findings are not only important for fundamental human neurobiology, but they also have a potential clinical importance as well: It is known that early brain activity of this kind in preterm infants in the NICU is highly sensitive to medical treatments or brain injury (Skov et al. 1992; Malk et al. 2013). This suggests that there might be possibility to follow and maybe intervene in optimizing brain activity, which has raised interest in the context of adjusting preterm care to improve brain growth and neurodevelopmental outcomes (Gale 2004; Ehrenkranz 2006; Yang et al. 2011). It is notable in this context that the early structural brain growth was mostly considered to be independent of external sensory experience (Bourgeois et al. 1989; see also reviews in Hanganu-Opatz 2010; Colonnese and Khazipov 2012). Yet, some studies in preterm infants have suggested associations between early interventions and later functional or structural outcomes (Als et al. 2004; Guzzetta et al. 2009; Milgrom et al. 2010).

Neurobiological Underpinnings of Early Structure–Function Relationship

Neurobiological interpretation of the present findings needs reconciliation of current knowledge about early development of neuronal networks on one hand, and the knowledge of histological underpinnings of MRI findings on the other hand. It is generally known that most neurons are already existing by the time our first MRI images were acquired after preterm birth, hence global neuronal proliferation and migration are unlikely to contribute to the observed volume increase, even though more recent studies have clearly outlined the protracted neuronal proliferation in humans even beyond birth in selected neuronal populations such as the GABAergic interneurons (Sanai et al. 2011; Malik et al. 2013). Other mechanisms potentially influencing volume growth of brain tissue during this developmental period are the growth and myelination of dendritic and axonal structures (Mrzljak et al. 1992; Petanjek et al. 2008; Kiss et al. 2014) while the increase in synapses has at most minor effects on brain volumes during preterm life (Huttenlocher and Dabholkar 1997; Petanjek et al.

2011). During initial stages of development, intrinsic experience-independent neuronal activity plays a critical role in network remodeling and may lead to growth or pruning of axonal or dendritic branches. Later, removal of extrinsic activity in critical periods leads to the well-known alterations of neuronal circuits and functional loss as shown in studies on visual deprivation in primates (Bourgeois et al. 1989) and cats (Shatz and Stryker 1988). This known developmental sequence suggests that the interpretation of our MRI findings is to be found from the mechanisms that link the early neuronal network activity to the survival and/or increase in neuronal arborization and glial cells.

A wide range of experimental and clinical studies have established that the survival of developing neurons is heavily dependent on the spontaneously arising activity in the early connected neuronal networks (Blankenship and Feller 2009; Hanganu-Opatz 2010; Colonnese and Khazipov 2012). Deprivation of neurons of this activity may readily lead to apoptotic cell death (Kilb et al. 2011; Nimmervoll et al. 2013) and pruning or elimination of their arbors (Catalano and Shatz 1998; Tolner et al. 2012). Recent studies on neonatal nonhuman primates have reported significantly increased apoptotic cell death of both glial and neuronal structures after administration of various anesthetic drugs (Brambrink et al. 2012) that reduce neuronal activity. These lines of evidence jointly point toward the idea that reduction of endogenous brain activity in the very early neuronal networks could lead to a relative loss, or decreased survival, of cellular structures (glia and neuronal dendrites and axons) that are jointly measured by structural MRI. It is interesting in this context that a recent observational study reported less brain maturation and volumes, and lower language scores at 2 years of age in infants cared for in private rooms than in open wards, possibly related to relative sensory deprivation (Pineda et al. 2014). However, our 2 examined biomarkers (brain waves and brain volume) are only very global measures and collected at remote time points, and caution is therefore needed in inferring neurobiological brain changes. In the near future, the emerging neuroimaging modalities for human in vivo studies hold promise for narrowing the gap between axonal microstructure or glial volumes (Assaf et al. 2013; Zhang et al. 2014) and brain activity. Cortical neuron differentiation and lamination are also under the control of growth factors, axon–cell interactions coming from the extracellular matrix, which is particularly prominent in the subplate during the developmental period under study (Kostović et al. 2014). These factors may well be indirectly linked to prematurity associated injury to the subplate and therefore indirectly linked to lower spontaneous activity (Kinney et al. 2012). Subplate neurons appear to function as a synaptic placeholder and form transient synapses with thalamic axons during the time of highest vulnerability to preterm brain injury (Kanold 2009).

It is hence fundamentally important to identify the human EEG counterparts of the brain activity that might provide the vital drive to neuronal survival and network growth. Recent studies in experimental animal models (Hanganu-Opatz 2010; Kilb et al. 2011) and in human neonates (Vanhatalo et al. 2005; Colonnese and Khazipov 2012) have provided evidence that the key functional driver of neuronal development comprises the intermittent events, SATs (Vanhatalo and Kaila 2006), and experimental work has shown how disruption of such early activity leads to disorganized thalamo-cortical connectivity and neuronal death (Catalano and Shatz 1998; Tolner et al. 2012). Comparison between preterm development of EEG activity and brain gyration and growth of pathways (Kostović and Judaš 2010) reveals a strikingly close temporal relationship (Khazipov and Luhmann 2006; Vanhatalo and Kaila 2006): The EEG waveforms become increasingly complex with gyration being compatible with the spread of activity over gyri and sulci, whereas spatial synchronies, both intra- and interhemispheric, increase jointly with the known growth of thalamocortical and cortico-cortical connections (Kostović and Judaš 2010). Our present work builds on these bridges by offering the first noninvasive metrics to probe the correlation between brain activity and growth in human babies.

Our event- and amplitude-based metrics were designed to capture those brain events and to generate robust metrics for their quantification. The significant correlation between higher brain activity levels and increased brain volumes, including subplate, is fully compatible with the idea that neuronal growth and survival is sensitive to early network activity (Nimmervoll et al. 2013). This is also confirmed by Natalucci et al. (2013), showing that early aEEG maturation score related to brain maturation on conventional MRI at term equivalent age.

More critical evaluation of relationships between brain activity and macro- and microstructure, consideration of perinatal development of synapses and better insights in genetic–epigenetic factors involved in brain growth will be promoted by these findings, initiating both basic science translational research and longitudinal research of structure and function in relation to the developing human brain in larger cohort of neonates.

Developmental Influence of Extra-Uterine Environment

Electroencephalography

The effect of the extra-uterine environment on brain development has been a matter of controversy for a long time, and many of the apparent discrepancies in the literature are likely related to the level of inspection, or varying interpretation of causalities. Indeed, it is very challenging if not impossible to prove causal effects of nonphysiological (i.e. extra-uterine) environment on a preterm baby brain activity, because the brain activity itself is strongly affected by cardiorespiratory homeostasis (West et al. 2006; Wikström et al. 2011), as well as by medical adversities and care procedures faced by most of the preterm babies. Systematic cohort studies have shown that extra-uterine preterm life often delays maturation of brain activity in ways that are especially apparent in the spatiotemporal organization of spontaneous EEG activity (reviewed in Tharp 1990). Some later studies have assessed the relationship between extra-uterine environment and individual EEG transients (Conde et al. 2005) or compressed EEG measures (Soubasi et al. 2009), and then speculated the findings to reflect an advanced maturation ex utero (Hafner et al. 1994; Klebermass et al. 2006). However, many such EEG changes may also be readily explained by the cardiovascular stabilization with advancing postnatal age, which in turn leads to an improved brain activity without direct relationship to the elusive level of brain maturation. The consequences of an early exposure to repeated stimuli on brain maturation are largely unknown, and need to be elucidated in combination with structural brain development assessed with MRI and long-term outcome (Bonifacio et al. 2010; Smith et al. 2011).

Magnetic Resonance Imaging

Gray matter volume and also myelination at term age (Hüppi et al. 1998; Inder et al. 2005) are affected by prematurity. The deep gray matter has recently gained interest because of the importance of thalamo-cortical development. The identification of deep gray matter growth failure is not an isolated phenomenon, but rather it is associated with the maldevelopment of remote structures. This could be mediated by a disturbance in thalamo-cortical connectivity during a critical period in cerebral development (Boardman et al. 2006). It is know that early synaptic activity in the developing cortex regulates axonal and dendritic growth and branching (Mire et al. 2012) leading to cortical network development formed by thalamo-cortical, cortico-cortical, and cortico-thalamic connectivity. In a recent study, whole brain connectome analysis in preterm infants of 6 years has shown weakened connectivity in the cortico-basal ganglia connections and increased strength in regional cortico-cortical connections (Fischi et al. 2014).

White matter injury in prematurity and prematurity per se is well known to affect the development of neural pathways (i.e. thalamo-cortical networks; Hüppi et al. 2001; Ball et al. 2012), and this might further affect cortical laminar and deep gray matter development resulting in overall brain volume loss (Volpe 2009; Kostović and Judaš 2010). In experimental animal models, it was recently demonstrated that hypoxic–ischemic injury and infection-induced inflammation in premature sheep brain result in a decreased number of dendritic branches and synapses and altered brain volume and microstructure (van de Looij et al. 2014). Therefore, prematurity associated injury may lead to loss of synapse-dependent activity in early development. BGTh might be particularly affected by disruption of synaptic activity in the subplate due to white matter injury that might lead to disrupted circuit development and deficient functions (Volpe 2009; Kostović and Judaš 2010).

There is only one study suggesting that maturation of brain structures may be accelerated by factors associated with preterm birth, measured by quantitative analysis of the fractional anisotropy maps using voxel-based morphometry obtained by diffusion tensor imaging (Giménez et al. 2008). Future studies need to reveal whether increased brain activity mediates these effects. In addition, measures of cortical folding, surface, and index at birth (Dubois, Benders, Cachia, et al. 2008) correlated positively with aEEG measures of higher activity in our study, and this supported earlier data that showed that surface and sulcation index at birth were related to neonatal behavioral performance in preterm newborns at term equivalent age, and that these measures were affected by changes in early intrauterine environment (Dubois, Benders, Borradori-Tolsa, et al. 2008). Future studies with more functional oriented imaging such as resting state-functional MRI may help to elucidate whether these comprise in relation to brain activity and neurodevelopmental outcome, or whether this occurs under genetic control.

Signal Analysis Considerations

Our current work introduces implementation of several EEG-derived parameters that can be readily automated even as a real-time index to be displayed in the brain monitors during neonatal intensive care (for considerations related to rEEG metrics, see O'Reilly et al. 2012). A successful clinical use of more sophisticated EEG analyses requires computational paradigms that are robust to common confounders, such as technical artifacts encountered in the NICU environment (Walls-Esquivel et al. 2007; Palmu et al. 2013). A particular advantage in our event-based EEG metrics is the opportunity for a direct translation between studies performed in humans and the experimental models, including in vivo preparations (Khazipov and Luhmann 2006; Vanhatalo and Kaila 2006; Seelke and Blumberg 2010; Colonnese and Khazipov 2012).

We feel that some specific characteristics of our current metrics warrant further attention with respect to their wider use in human infant studies. All measures of single event, such as IBImax that measures the longest IBI in the EEG recording inspected, are conveniently measured and they have been popular in the past literature (Hayakawa 2001; Wikström et al. 2012). However, the power law scaling of brain events (Roberts et al. 2014) means that the likelihood of a single very long IBI increases with the length of data, and hence the maxIBI is influenced by both data length and detector settings. Moreover, any measure based on single event, whether activity or inactivity, is critically sensitive to the presence of artifacts. Such measures should rather be analyzed with respect to their statistical properties, for example by taking a given percentile level, or median of a larger sample. Same limitation does also apply to measures that identify the data segment with lowest or highest values, such as the SAT#min and SAT%min used in our study. Even a relatively short epoch with strongly deviant brain activity (e.g. few minutes after drug injection; cf. Skov et al. 1992) may confound the whole metrics that is recorded to inform about infant's brain wealth in the longer term. In order to overcome these challenges, we complemented our study by taking the average SAT% over a continuous 2.5-h period, which is least sensitive to transient technical or biological confounders. A long-term average or median value of event-based metrics would likely be technically most reliable and a stable candidate value in future clinical trials that aim to validate these measures as early functional biomarkers.

Finally, it should be noted that the above technical and conceptual challenges associated with the EEG analyses are negligible compared with the resources invested in the development of modern neonatal neuroimaging techniques. The current routine EEG analysis tools of basic science laboratories can be readily transferred into clinical environment for an objective assessment of brain activity in the human preterm babies.

Conclusions

Early postnatal evaluation of brain maturation using novel metrics of long-term EEG signal can be used to study the correlation with structural brain growth during preterm brain development. Our observations with the limited patient series are consistent with the idea that increased levels of endogenous or exogenous brain network activity are associated with better brain growth. Such observation is fully compatible with the recent experimental work, showing the intimate relationship between structure and function in early brain development. The EEG and MRI metrics employed in our work provide putative biomarkers to be used in parallel with translational studies that involve both human babies and experimental animal models (Kiss et al. 2014; van de Looij et al. 2014). Finally, the development of objective, real-time monitoring paradigms holds promise for guiding individually optimized NICU care and early neurodevelopmental interventions (www.eadcare.org; Als et al. 2004; Guzzetta et al. 2009).

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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Notes

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