

# Changes in brain morphology and microstructure in relation to early brain activity in extremely preterm infants

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**BACKGROUND AND OBJECTIVE:** To investigate the relation of early brain activity with structural (growth of the cortex and cerebellum) and white matter microstructural brain development.

**METHODS:** A total of 33 preterm neonates (gestational age  $26 \pm 1$  weeks) without major brain abnormalities were continuously monitored with electroencephalography during the first 48 h of life. Rate of spontaneous activity transients per minute (SAT rate) and inter-SAT interval (ISI) in seconds per minute were calculated. Infants underwent brain magnetic resonance imaging  $\sim 30$  (mean 30.5; min: 29.3–max: 32.0) and 40 (41.1; 40.0–41.8) weeks of postmenstrual age. Increase in cerebellar volume, cortical gray matter volume, gyrification index, fractional anisotropy (FA) of posterior limb of the internal capsule, and corpus callosum (CC) were measured.

**RESULTS:** SAT rate was positively associated with cerebellar growth ( $P=0.01$ ), volumetric growth of the cortex ( $P=0.027$ ), increase in gyrification ( $P=0.043$ ), and increase in FA of the CC ( $P=0.037$ ). ISI was negatively associated with cerebellar growth ( $P=0.002$ ).

**CONCLUSIONS:** Increased early brain activity is associated with cerebellar and cortical growth structures with rapid development during preterm life. Higher brain activity is related to FA microstructural changes in the CC, a region responsible for interhemispheric connections. This study underlines the importance of brain activity for microstructural brain development.

The preterm brain is highly vulnerable in the first postnatal weeks of life, and maintaining structural and functional brain integrity during this critical period is one of the main challenges in neonatal health care. The assessment of early brain activity is an important biomarker of functional brain development in preterm-born neonates (1,2). In early life myelination of the thalamocortical axons is guided by spontaneous electrical signals, and electroencephalography

(EEG) is a useful tool for the assessment of the functional status of these connections. The filtered and time-compressed amplitude-integrated EEG (aEEG) is a bedside tool available for continuous monitoring of brain function in the neonatal unit. Increased brain activity on aEEG is recognized as bursts of high voltage with rapid oscillations, interrupting a period of inactivity (3,4). These high voltage bursts are known as spontaneous activity transients (SATs) and are thought to be crucial for the establishment of brain networks (1). SATs have unique and specific characteristics and can be used as representation of activity in different brain areas and they are necessary for a normal brain. In somatosensory regions, SATs are associated with limb movements in mammalian fetuses and infants (5). In the primary visual cortex, SATs are the expression of spontaneous retinal waves, and are abolished by eye removal (6). In humans, visual SATs abruptly disappear  $\sim 35$  weeks of gestation (7). A previous study in our center showed that increased electrical brain activity, or less cortical electrical quiescence, in the first postnatal days correlated with faster volumetric growth of different brain structures up to term equivalent age (TEA) (8). The hypothesized correlation of brain functional and structural maturation creates an early potential interesting predictor of altered brain development.

However, the effect of early brain activity on microstructural brain development has not been established. Fractional anisotropy (FA) is related to fiber coherence, axonal density, and degree of white matter organization. FA of the white matter typically increases with brain maturation and correlates with long-term neurodevelopment (9,10).

White matter, cerebral cortex, and cerebellum are the largest and most vulnerable brain structures in extremely preterm neonates. Furthermore, a developmental relation is suggested between the cerebral cortex and the white matter (11), as well as between the cerebral cortex and the cerebellum (12). The aim of this study was to examine the structural development of the cerebral cortex and cerebellum of the

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preterm brain, as well as the microstructural development of white matter in relation to early brain activity.

## MATERIALS AND METHODS

### Study Population

All neonates with a gestational age (GA) at birth below 28 weeks, born between May 2008 and March 2013, and admitted to the Neonatal Intensive Care Unit of the Wilhelmina Children Hospital (Utrecht, The Netherlands) received, according to clinical protocol, aEEG monitoring during the first three days of life and magnetic resonance imaging (MRI) of the brain ~30 weeks postmenstrual age (PMA), and again around TEA. Infants with congenital malformations, genetic disorders, or metabolic diseases were excluded. Infants with severe brain injury ( $n=20$ ) were also excluded, as severe brain injury is known to affect the reliability of MRI analysis. Morphine administration is also known to interfere with early brain activity (13). Assessing this effect was beyond the scope of this study, and therefore neonates receiving morphine during aEEG registration were excluded as well ( $n=10$ ). The total number of inclusions and exclusions can be found in **Supplementary Figure S1** online. The medical ethical review committee gave permission for use of the clinical data for research purposes.

### Clinical Parameters

Clinical parameters were obtained by chart review. Birth weight (BW)  $z$ -scores were computed using the Dutch population reference data (14). Bronchopulmonary dysplasia was considered present in case of oxygen dependency at 36 weeks of GA. Hypoglycemia was defined as a whole-blood glucose level  $<2.5$  mmol/l. Hypotension was defined as mean arterial blood pressure (mm Hg) of less than the GA (in weeks) at that time. Arterial blood pressure was measured using an indwelling arterial catheter. Severe brain injury (an exclusion criterion) was defined as the presence of an intraventricular hemorrhage grade III or IV (according to Papile *et al.* (15)), posthemorrhagic ventricular dilatation (ventricular index  $>97$ th percentile) (16), periventricular leukomalacia grade II or III (17), or a large cerebellar hemorrhage ( $>3$  mm). White matter injury was assessed by two neonatologists (L.S.d.V. and M.J.N.L.B.) using the Kidokoro scoring system (18).

### aEEG Acquisition

Bedside aEEG at a sampling rate of 256 Hz was started as soon as possible after birth and continued for at least 48 h. Subcutaneous needle electrodes were used with a central reference electrode measuring the impedance. The P3–P4 cross-sectional signal was chosen, and is known to be predictive of neurodevelopment in preterm infants (19). Owing to technical reasons (impedance, inhomogeneity of data, and differences in filters) only infants monitored with BrainZ monitors (BRM2/BRM3, BrainZ; Natus, Seattle, WA) were enrolled (**Supplementary Figure S1**).

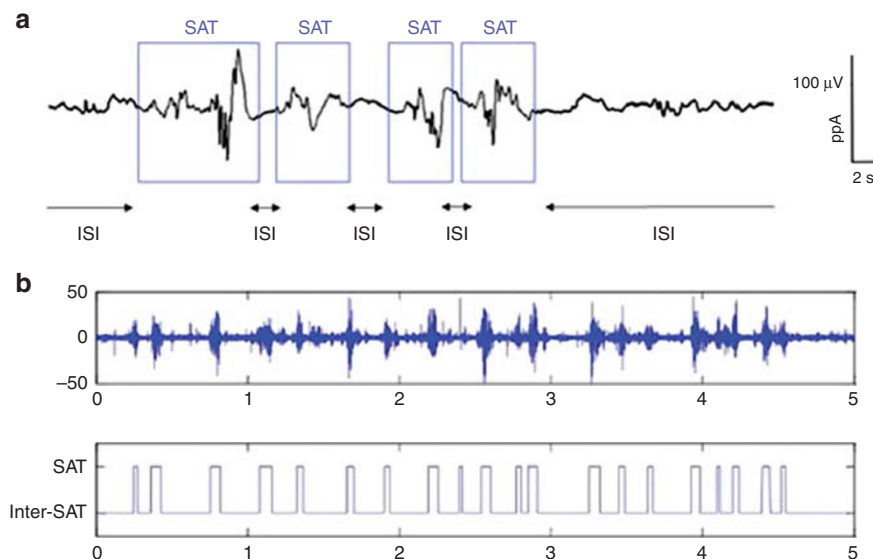
### EEG Postregistration Analysis

In-house-developed software (SignalBase v7.8, University Medical Center, Utrecht, The Netherlands) was used to process the raw EEG data. In all patients three epochs of 1 h were manually selected at the aEEG at three specific postnatal time points: 20–24 h (T1), 32–36 h (T2), and 44–48 h (T3). The aEEG records were visually assessed to identify artifacts, caretaking events, and periods with high impedance, to select the best hour per epoch.

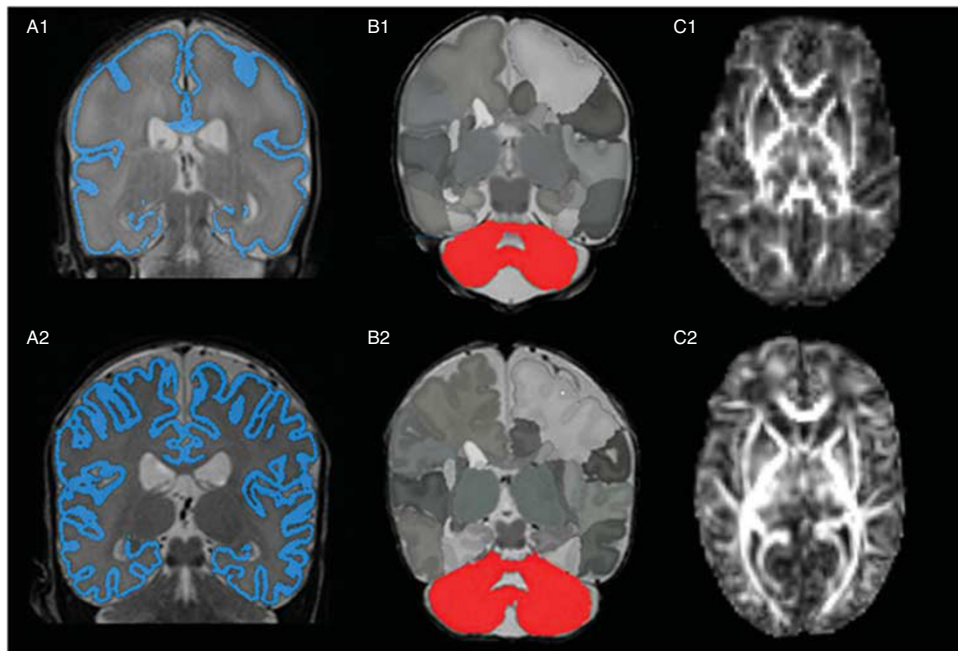
Quantitative analysis with the same software program obtained the number of SATs per minute (SAT rate) (rounded to whole number) and the inter-SAT interval (ISI, i.e. time between SAT) in seconds per minute, both derived from the raw EEG. SAT and ISI, the so-called event-based EEG measures, are explained in **Figure 1a, b**. The quantification of SAT and ISI was done using a nonlinear energy operator (<http://iopscience.iop.org/0967-3334/31/11/N02>). In the present cohort, seven patients were registered with BRM 2 and 26 patients with BRM 3 monitors. These devices had a filter setting of 2 Hz and 0.5 Hz, respectively, which implied a lower sensitivity of BRM 2 monitors for low frequencies. Ten patients monitored with BRM3 were randomly selected and resampled with a 2 Hz filter. The results were compared with the 0.5 Hz filter and no significant differences were found in EEG parameters.

### MRI Acquisition

MRI was performed on a 3T MR system (Achieva; Philips Medical Systems, Best, The Netherlands). At 30 weeks, PMA, an MRI-compatible incubator, was used to reduce patients discomfort and maintain temperature (Dräger MR Incubator, Lübeck, Germany and Nomag IC 3.0, Lammers Medical Technology GmbH, Lübeck, Germany, with a dedicated neonatal head coil). At TEA, infants were positioned within a vacuum pillow in a SENSE head coil. The protocol included T2-weighted imaging in the coronal plane (turbo



**Figure 1.** Event-based electroencephalography (EEG) measures. (a) An example of a 30 s EEG record of one infant showing several spontaneous activity transients (SATs) with the inter-SAT intervals in between (ISI). (b) An epoch of 5 min EEG with below detected SAT and ISI.



**Figure 2.** Examples of image analysis results. The top row shows the 30 weeks examples, the bottom row shows the 40 weeks examples. **(a1 and a2)** Examples of the method of Moeskops *et al.* (16) used for the cortical quantification analysis. **(b1 and b2)** Examples of the method of Makropoulos *et al.* (14) used to obtain the cerebellar volume. **(c1 and c2)** Examples of the diffusion tensor imaging (DTI) registration (<http://www.exploredti.com/>) used to obtain the fractional anisotropy (FA) of the posterior limb of the internal capsule and corpus callosum.

spin echo, at 30 weeks: repetition time 10,085 ms; echo time 120 ms; slice thickness 2 mm, in-plane spatial resolution  $0.35 \times 0.35 \text{ mm}^2$ ; at TEA: repetition time 4,847–6,293 ms; echo time 120–150 ms; slice thickness 1.2 mm, in-plane spatial resolution  $0.35 \times 0.35 \text{ mm}^2$ , full brain coverage) and diffusion tensor imaging (DTI) in the axial plane (single-shot spin-echo, repetition time 5,685 ms, echo time 70 ms, field of view  $180 \times 146 \text{ mm}^2$ , 32 diffusion-weighted images with  $B$  value of  $800 \text{ s/mm}^2$ , and one non-diffusion-weighted image; matrix size  $128 \times 102 \text{ mm}^2$ , slice thickness 2 mm, full brain coverage). During the whole examination a neonatologist was present. Oxygen saturation, respiratory, and heart rate of the infants were monitored. If necessary, the infants were sedated using oral chloral hydrate 30 mg/kg at 30 weeks PMA and 50–60 mg/kg at TEA. All infants received double-layer hearing protection using Minimuffs (Natus Medical Incorporated, San Carlos, CA) and Earmuffs (EM's 4 Kids, Brisbane, QLD, Australia).

### MRI Analysis

T2-weighted scans were used to segment the cerebellum with an automated segmentation method (20), which allowed computation of cerebellar volume. This method has shown reliable results among infants scanned between 28 and 44 weeks PMA. Results of all segmentations were visually inspected and small corrections were performed if deemed necessary. Examples are provided in **Figure 2b**.

To enable analysis of the cortex, another automated segmentation method was used for the segmentation of the cortical gray matter using T2-weighted images (21). This method allowed computation of cortical gray matter volume ( $\text{cm}^3$ )—characterizing cortical growth—and gyrification index—characterizing cortical maturation (22). Gyrification index was measured as the ratio between inner cortical surface and a smooth convex hull around the white matter (i.e., a simulated cortex around the brain without any folds). Example results of cortical gray matter segmentations are shown in **Figure 2a**.

Quality assessment and analysis of DTI images were performed with the diffusion MRI toolbox *ExploreDTI* (<http://www.exploredti.com/>). Data were corrected for eddy current-induced geometric distortions and subject motion as described previously (23). In this

procedure, the required reorientation of the B-matrix was performed and the tensor model was fitted to the data with the REKINDLE approach (23,24). The FA values of the posterior limb of the internal capsule (PLIC) and corpus callosum (CC) were computed by registering the images to a neonatal atlas (25) using both affine and elastic transformation (**Figure 2c**).

### Statistical Analysis

Statistical analysis was performed using IBM SPSS v21.0 (Chicago, IL). The time effect of EEG variables over the three epochs (T1–T2–T3) was checked through a linear regression model, and in the absence of time dependency the three epochs were averaged to a single value of SAT rate and ISI. All structural brain volumes at 30 weeks and at TEA were corrected for total brain volume. Growth of the structural brain volumes and increase of gyrification index and FA parameters were measured as growth/increase per 10 weeks. For these calculations, the absolute difference between the measurement at TEA and at 30 weeks of GA was taken, divided by the period (in weeks) between the two scans and multiplied by 10 with the following formula:  $((\text{vol } 40 - \text{vol } 30) / \text{weeks between scans}) \times 10$ , as already described by Kersbergen *et al.* (26). All data were corrected for PMA at the time of the MRI. First, a univariable regression analysis was performed to determine which factors had the largest effect on MRI parameters (results shown in **Supplementary Table S1**). Owing to the small sample size, only the most influential variables were included in the multivariable linear regression analysis. A univariable linear regression analysis was used to determine the effect of clinical confounders on EEG variables (results shown in **Supplementary Table S2**). The multivariable regression model included GA, BW  $z$ -score, and white matter injury score and was built to evaluate the associations between quantitative measures of early brain activity and increase of the MRI measures. A separate multivariable model was built per EEG parameter and MRI parameter. A  $P$  value below 0.05 was considered statistically significant.



**Table 1.** Baseline characteristics of the study population

Baseline characteristics	N=33
Gestational age (weeks), mean (SD)	26.0 (1.0)
Birth weight, mean (g) (SD)	916 (157)
SGA (<p10), n (%)	2 (6)
Male/female, n (%)	12/21 (36/64)
Apgar score 1 min, median (IQR)	6 (3–7)
Apgar score 5 min, median (IQR)	8 (7–9)
pH arterial/venous cord blood, mean (SD)	7.27 (0.08)
<i>Hypoglycemia (&lt;2.5 mmol/l)</i>	
Once, n (%)	4 (12.1)
Repeated episodes, n (%)	1 (3)
<i>Hypotension (MABP&lt;GA)</i>	
Treatment: fluids, n (%)	4 (12.1)
Inotropes, n (%)	12 (36.4)
GMH-IVH grade I–II, n (%)	3 (9)
<i>Patent ductus arteriosus</i>	
Conservatively treated, n (%)	16 (49)
Surgically treated, n (%)	1 (3)
Necrotizing enterocolitis, n (%)	1 (3)
Bronchopulmonary dysplasia, n (%)	5 (15)
Culture-proven sepsis, n (%)	14 (42)
Punctate cerebellar hemorrhage at TEA, n (%)	3 (9)
Mild or moderate WMI, n (%)	23 (77)
Weight at 30 weeks scan, mean (g) (SD)	1,183 (190)
HC at 30 weeks scan, mean (cm) (SD)	27 (2)
Weight at 40 weeks scan, mean (g) (SD)	3,421 (388)
HC at 40 weeks scan, mean (cm) (SD)	35 (1)

GA, gestational age; GMH, germinal matrix hemorrhage; HC, head circumference; IQR, interquartile range; IVH, intraventricular hemorrhage; MABP, mean arterial blood pressure; SGA, small for gestational age; TEA, term equivalent age; WMI, white matter injury.

## RESULTS

### Patients

In total, 33 infants were eligible for the study, all with good quality aEEG and serial MRI. Clinical characteristics are presented in **Table 1**. Early MRI was performed at a mean PMA of 30.5 (range 29.3–32.0) weeks and TEA MRI at a mean PMA of 41.1 (range 40.0–41.8) weeks. Growth of the structural and microstructural brain measurements between 30 and 40 weeks is shown in **Figure 3**. None of the enrolled patients were presented with clinical or laboratory signs of perinatal asphyxia. None of the infants were mechanically ventilated during EEG monitoring. One infant received phenobarbital at the dose of 10 mg/kg on day 2 because of suspicion of electrical discharges. However, no severe brain injury was observed in this patient. None of the other

included infants received any other medication influencing brain activity such as benzodiazepines and barbiturates.

In infants with clinical signs of hypotension needing inotropes, the mean dopamine dose was 5 µg/kg/min, SD 2.3, range 5–12 µg/kg per min. The univariable linear regression analysis on the effect of clinical confounders on EEG variables showed that BW z-score was significantly and negatively associated with ISI. (**Supplementary Table S2**).

### Early Brain Activity and Structural Brain Growth

A significant positive association between SAT rate and cerebellar growth was found with conversely a significant negative association between ISI and cerebellar growth (**Table 2** and **Figure 4**). Other clinical variables did not show an effect to cerebellar growth in the multivariable analyses. SAT rate also was positively correlated with growth of cortical gray matter and increase in gyrification index, where ISI did not show any relation.

A clear example of the clinical relevance of the present study is that an increase of one SAT/min in the first 48 h after birth will bring an increase of ~0.2 cm<sup>3</sup> to the cerebellar volume and of ~5 cm<sup>3</sup> of cortical gray matter volume. An increase of one second in ISI will result in a decrease of ~0.14 cm<sup>3</sup> in cerebellar volume.

### Early Brain Activity and Microstructural Brain Growth

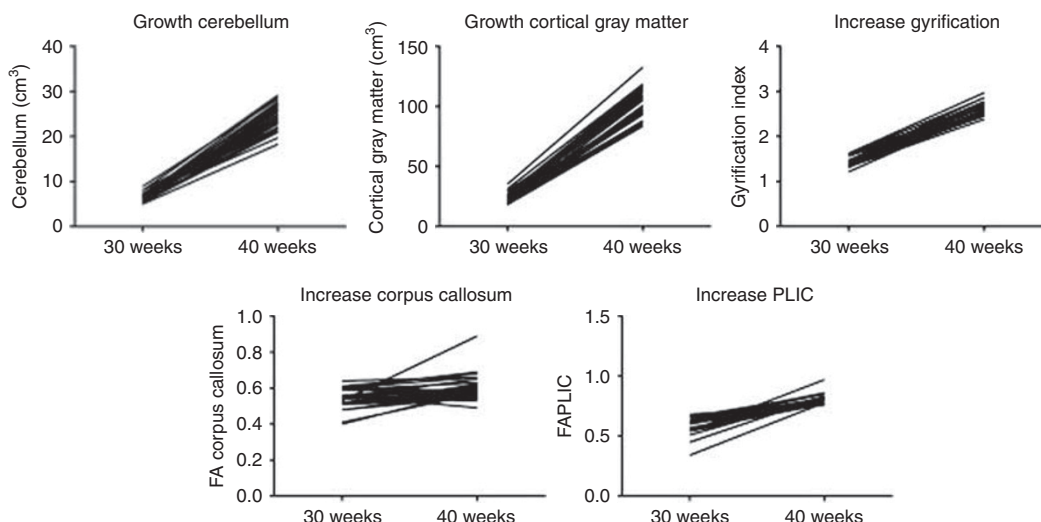
The multivariable analysis showed a positive association between SAT rate and increase in FA of the CC (**Table 3**). Thus, an increase in one SAT per minute will result in an increased FA value of the CC of ~0.03.

No association between ISI and the CC was seen. Also, no associations were found between EEG measurements (SAT and ISI) and increase in FA of the PLIC.

## DISCUSSION

This study shows increased early brain activity (expressed by higher SAT rate and lower ISI) to be associated with increased growth of the cerebral cortex and cerebellum and also with changes in corpus callosal FA, in a relatively healthy cohort of extremely preterm infants. These results underline the importance of early life neuromonitoring in extremely preterm neonates in order to identify those at risk of altered brain development.

This study confirms the importance of SATs for cortical gray matter growth, as presented in a previous paper (8) and adds the importance of early spontaneous activity to cortical maturation (i.e., gyrification). SATs are the most notable feature of the preterm EEG, characterized by nested activity at multiple frequencies, including also very-low-frequency activity (0.1–0.5 Hz) that is filtered by conventional EEG (1). In healthy human infants, SATs disappear ~35 weeks of PNA (27). The association between early brain activity (i.e., SAT rate and ISI) and morphological maturation of the cortex between 30 and 40 weeks is thought to be a result of the complex interplay between structural and functional processes in this period of brain network development (28). EEG



**Figure 3.** Structural and microstructural brain growth. Increase between 30 and 40 weeks for all 33 individuals are shown for cerebellar volume, cortical gray matter (cGM) volume, gyrification index (GI), corpus callosum (CC) fractional anisotropy (FA), and posterior limb of the internal capsule (PLIC) FA. For each variable mean (SD) and range (i) is provided: cerebellum 30 weeks (cm<sup>3</sup>): 6.48 (0.87) (5.00; 8.90), 40 weeks: 24.56 (2.63) (18.28; 29.16). cGM 30 weeks (cm<sup>3</sup>): 22.84 (3.91) (17.78; 35.18), 40 weeks: 104.57 (11.82) (83.65; 132.69). GI 30 weeks: 1.40 (0.09) (1.21; 1.62), 40 weeks: 2.66 (0.13) (2.38; 2.97). FA CC 30 weeks: 0.53 (0.06) (0.40; 0.64), 40 weeks: 0.59 (0.08) (0.36; 0.89). FA PLIC 30 weeks: 0.57 (0.07) (0.34; 0.68), 40 weeks: 0.80 (0.04) (0.69; 0.97). All the values were corrected age at scan.

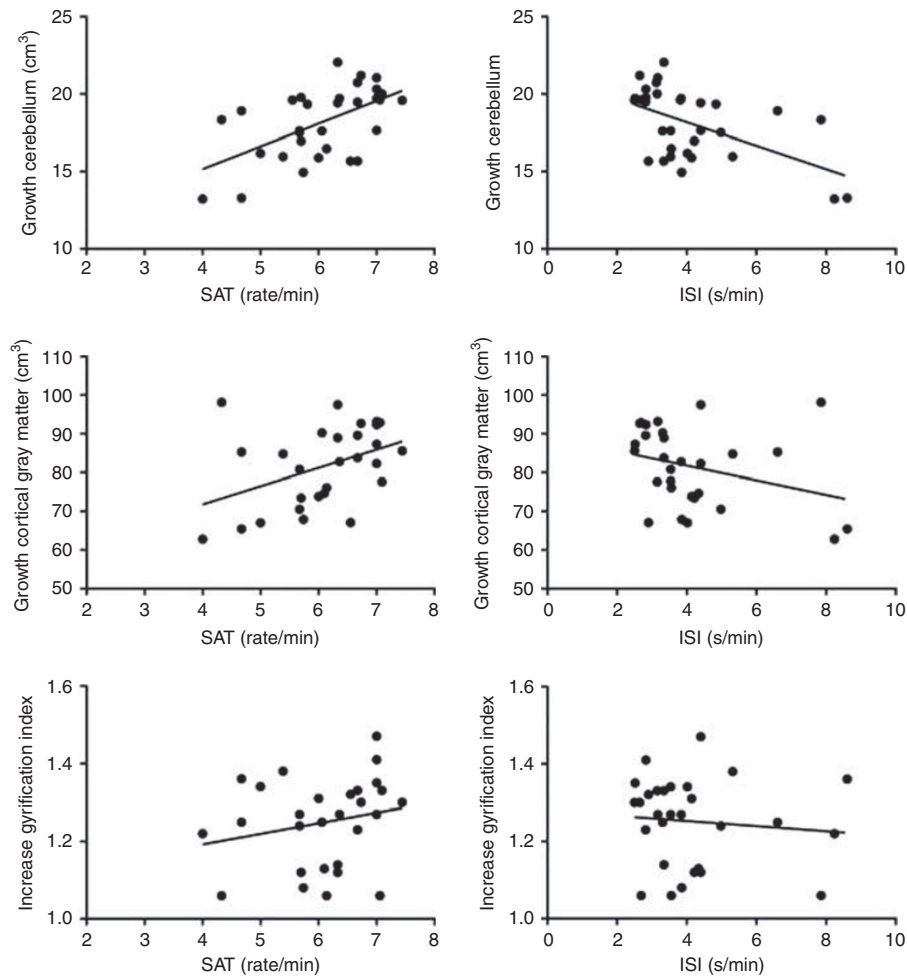
**Table 2.** EEG parameters and structural brain development

	Cerebellum		Cortical gray matter			
	Volumetric growth, <i>B</i> (CI)	<i>P</i> value	Volumetric growth, <i>B</i> (CI)	<i>P</i> value	Gyrification, <i>B</i> (CI)	<i>P</i> value
SAT	<b>0.234 (0.060; 0.408)</b>	<b>0.010</b>	<b>4.706 (0.566; 8.847)</b>	<b>0.027</b>	<b>0.050 (0.002; 0.099)</b>	<b>0.043</b>
BW z-score	-0.008 (-0.255; 0.239)	0.945	0.702 (-4.598; 6.002)	0.787	<b>-0.079 (-0.140; -0.018)</b>	<b>0.014</b>
WMI score	0.040 (-0.093; 0.173)	0.540	-1.134 (-4.498; 2.231)	0.493	-0.032 (-0.070; 0.006)	0.095
GA	0.022 (-0.152; 0.195)	0.801	1.356 (-2.836; 5.548)	0.666	0.022 (-0.029; 0.073)	0.374
ISI	<b>-0.149 (-0.237; -0.062)</b>	<b>0.002</b>	-1.735 (-4.049; 0.578)	0.135	-0.019 (-0.047; 0.009)	0.178
BW z-score	-0.100 (-0.310; 0.109)	0.334	0.011 (-6.886; 6.907)	0.997	<b>-0.066 (-0.132; -0.001)</b>	<b>0.048</b>
WMI score	-0.020 (-0.150; 0.110)	0.753	-1.134 (-4.498; 2.231)	0.493	-0.022 (-0.060; 0.016)	0.238
GA	0.020 (-0.162; 0.202)	0.827	0.626 (-4.184; 5.435)	0.790	0.021 (-0.033; 0.074)	0.426

BW, birth weight; CI, confidence interval; EEG, electroencephalography; GA, gestational age; ISI, inter-SAT interval; SAT, spontaneous activity transient; WMI, white matter injury. Rows show the EEG parameters (SAT and ISI) and the other clinical parameters included in the multivariable analyses. Separate multivariable analyses were performed for ISI and SAT. Columns show the growth of cerebellar volume, cortical gray matter volume, and gyrification index. *B* value with CI and *P* value are shown. Volumetric growth is calculated per 10 weeks. Significant results are in bold.

findings are the result of cortical network activity, changing with maturation of the brain during the last part of gestation (29). From 24 weeks onward afferent thalamocortical axons grow from the thalamus into the subplate where accumulation and waiting take place before migration to the cortex. Migration from the subplate into the cortical plate induces synaptogenesis of the thalamocortical axons, which corresponds with the appearance of SATs at EEG (30,31). It is known that SATs are first focal and concentrated in the sensory cortex, and become more widespread later during development (1). Onset of synchronicity between hemispheres can be seen ~30 weeks of gestation, whereas more precise and consistent synchrony appears only from

~35 weeks of conceptional age, and it is not completely dependent on callosal connections (32). This study shows increased SAT rate to be associated with cortical gray matter maturation, which is supported by other studies finding more complex EEG patterns with increased gyrification (33). A recent study comparing subjects with no or minimal brain injury to those with moderate/severe brain injury showed that the latter have lower burst frequency and longer IBI and includes data from both rodent and human preterm subjects. In the rodent model of the same study, diminished brain activity was associated with a delayed neuronal morphological development demonstrating that spontaneous brain activity (SATs) is fundamental for normal maturation and



**Figure 4.** Electroencephalography (EEG) and structural brain growth. Associations of EEG parameters (spontaneous activity transient (SAT) and inter-SAT interval (ISI)) with cerebellar volume, cortical gray matter volume, and gyrification index are shown. Coefficients and confidence intervals corrected for confounding factors are shown in [Table 2](#); therefore, *R* and *P* values are not included in this figure. EEG variables mean (SD) and range (:) are provided as follows: SAT (rate/min): 6.0 (0.8) (4.0;7.4), ISI (s/min): 4.1 (1.6) (2.5;8.6).

**Table 3.** EEG parameters and microstructural brain development

	Corpus callosum		PLIC	
	Increase FA, <i>B</i> (CI)	<i>P</i> value	Increase FA, <i>B</i> (CI)	<i>P</i> value
SAT	<b>0.027 (0.020; 0.053)</b>	<b>0.037</b>	-0.013 (-0.067; 0.042)	0.608
BW z-score	0.004 (-0.051; 0.059)	0.882	-0.054 (-0.120; 0.012)	0.098
WMI score	-0.012 (-0.052; 0.027)	0.501	<b>-0.105 (-0.184; -0.027)</b>	<b>0.013</b>
GA	0.021 (-0.010; 0.052)	0.168	0.053 (-0.012; 0.117)	0.098
ISI	-0.008 (-0.022; 0.005)	0.207	0.009 (-0.018; 0.037)	0.466
BW z-score	0.006 (-0.052; 0.065)	0.811	-0.054 (-0.120; 0.012)	0.098
WMI score	-0.009 (-0.051; 0.033)	0.639	<b>-0.105 (-0.184; -0.027)</b>	<b>0.013</b>
GA	0.030 (-0.001; 0.060)	0.053	0.053 (-0.012; 0.117)	0.098

BW, birth weight; CI, confidence interval; EEG, electroencephalography; FA, fractional anisotropy; GA, gestational age; ISI, inter-SAT interval; PLIC, posterior limb of the internal capsule; SAT, spontaneous activity transient; WMI, white matter injury.

Row show the EEG parameters (SAT and ISI) and the other clinical parameters included in the multivariable analyses. Separate multivariable analyses were performed for ISI and SAT. Columns show increase of fractional anisotropy FA in the corpus callosum and PLIC. *B* value with CI and *P* value are shown. Microstructural brain development was calculated as growth per 10 weeks. Significant results are in bold.

development of cortical circuits and neurons. These data suggest that early brain injury impairs activity-dependent maturation of neuronal circuits during a highly sensitive period of development (34). We were able to show that increased cerebellar growth is associated with early brain activity, whereas in contrast, low SAT rate and prolonged ISI are associated with poor cerebellar growth. An approximately three times increase in cerebellar volume from 28 to 40 weeks of gestation has been documented, which makes the cerebellum, more than the cortical gray matter, the brain structure showing the largest relative growth in this period (26). This suggests the cerebellar and cortical expansion to be most vulnerable to disturbances (in brain activity) in this early period of life of preterm infants. Several studies have suggested a developmental relation between the cerebellum and cortical gray matter in infants born preterm, where the cortex and cerebellum appear to be connected in a closed circuit with bidirectional feedback, supported by recent studies describing reduced contralateral cortical gray matter growth in case of prematurity-related cerebellar injury (35–37). Both cerebellar and cortical alterations are associated with neurodevelopmental sequelae in the domains of cognition and motor development (35–37). This study, suggesting developmental dependency upon early brain activity of the cerebellum and the cortex, puts forward that cortical alterations might already find their origin in the first period of life in extremely preterm neonates. It cannot be excluded that the importance of brain activity and subsequent brain development is a surrogate of the infant's well-being, such as optimal cardiovascular and nutrition conditions, therefore optimal brain growth.

In this study, we showed prolonged ISI in early life to be associated with decreased cerebellar growth between 30 and 40 weeks. Prolonged ISI (and thus decreased cortical activity) has been associated with acidosis and adverse long-term neurodevelopmental outcome at 2 years of corrected age in preterm infants (19,38). Furthermore, some studies demonstrated that the survival of developing neurons is strongly dependent on the growing spontaneous activity in the early neuronal networks (39,40). Thus, deprivation of neurons of this activity, reflected by prolonged ISI, may be linked to cell apoptosis and reduced dendritic pruning (41,42). Based on our study, we can hypothesize that prolonged ISI in early life has more effect on cerebellar growth than on cortical gray matter development (43).

To the best of our knowledge, we have shown for the first time that increased early brain activity was associated with microstructural changes in the CC, as determined by faster increase in FA. This confirms the hypothesis of a link between early brain function and white matter microstructural maturation. White matter FA increases with brain maturation as a result of increasing myelination, fiber coherence, and axonal density. White matter organization is known to be correlated with long-term neurodevelopmental outcome in this vulnerable group of neonates (44,45). Being the largest white matter fiber bundle, the CC is responsible for the majority of the corticocortical, interhemispheric connections (46). Reduced FA of the CC might reflect altered myelination as well as axonal

damage, resulting in decreased interhemispheric processing. The PLIC is one of the WM structures with the largest increase in FA over the period of 30–40 weeks of PMA (47). White matter organization of the PLIC, predictive of motor development, and vulnerable to injury in preterm infants (44) did not show an association with early brain activity in this study. This could be explained by the fact that neonatal morbidity has a stronger effect on the process of maturation of the PLIC, than the effect found by early brain activity.

This study examined a relatively healthy cohort of preterm infants by excluding those who died, were unstable for MRI, received morphine, or had severe brain injury.

The main problem of these neonates is extreme prematurity, and many factors can influence later brain development. This study aimed to take into account as less severe preterm problems as possible by excluding infants with severe brain injury. In this group of vulnerable infants an association between early cortical network activity and brain microstructural development is suggested.

The first limitation of this study is the small population, leading to limited statistical strength. The analysis methods used require high-quality EEG and MRI data, resulting in a limited number of infants eligible for inclusion. However, selection bias does not seem to play a role, as baseline characteristics between included and excluded patients (based on image quality) were comparable. The small sample size results in a limited number of included relevant covariables in the analysis. Thus, the analysis could not be corrected for all confounders on aEEG and could not optimally be controlled for all known variables associated with brain growth. Furthermore, the small sample size yields the possibility to underscore the effect of other confounders.

## CONCLUSIONS

This study demonstrates the association of early brain activity to structural and microstructural brain growth in a cohort of relatively healthy extremely preterm infants. Higher SAT rate and decreased ISI are signs of increased early cortical network activity and appear to be associated with increased growth of cerebellum and cortical gray matter, as also cortical maturation. Cerebellum and cortex undergo the most rapid growth in the last part of gestation and are connected in a closed neuronal system. A change in FA of the CC was also shown to be associated with higher spontaneous brain activity in early preterm life. This study underlines the importance of early brain activity for improved microstructural brain development in extremely preterm infants and the need for neuromonitoring in these vulnerable newborns.

## SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/pr>

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