



EEG profiles and associated neurodevelopmental outcomes after very preterm birth



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HIGHLIGHTS

- Very preterm birth is associated with altered patterns of brain oscillations during adolescence.
- Altered oscillatory patterns were related to poorer intelligence and academic and motor skills.
- Specific oscillatory patterns may serve as biomarkers for poor outcome after very preterm birth.

ABSTRACT

Objectives: To assess brain oscillations in very preterm and full-term born adolescents and explore subgroups based on integrative patterns of brain oscillations with different frequencies. Additionally, subgroups were related to functional outcomes and very preterm birth.

Methods: A Dutch cohort of 53 very preterm and 61 full-term born adolescents aged 13 years participated. Resting-state electroencephalography was recorded. Absolute and relative delta, theta, alpha, and beta power were compared between groups. Latent class analysis (LCA) was used to identify subgroups with distinct activity patterns and to relate these patterns to intelligence, academic performance, motor skills, and very preterm birth.

Results: Very preterm adolescents exhibited decreased relative beta activity. LCA with four classes showed the best model fit. The class with the highest level of delta and lowest levels of theta, alpha, and beta power was associated with lowest intelligence, academic, and motor estimates. Very preterm born adolescents were more likely than controls to be members of this class.

Conclusion: Very preterm born adolescents showed altered oscillatory activity, indicating long-lasting effects of very preterm birth on brain functioning. Distinct activity patterns were associated with both functional outcomes and very preterm birth.

Significance: Specific patterns of brain oscillations may serve as biomarkers for poor functional outcome after very preterm birth.

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1. Introduction

Very preterm birth (<32 weeks of gestation) is associated with an increased risk for brain injury (Volpe, 2009) and long-lasting structural brain alterations (De Kieviet et al., 2012, Nosarti et al., 2014). These brain abnormalities are reflected in the wide range of neurodevelopmental impairments that are observed in very

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preterm born children (Aarnoudse-Moens et al., 2009, De Kievit et al., 2009, Twilhaar et al., 2018a, Twilhaar et al., 2018c). However, the neuronal mechanisms underlying these impairments are yet poorly understood. Despite advances in neonatal health care, long-term outcomes of very preterm born children have not improved over the last decades (Cheong et al., 2017, Burnett et al., 2018, Spittle et al., 2018, Twilhaar et al., 2018c). Importantly, high heterogeneity is observed across and within study samples (Twilhaar et al., 2018c). In order to improve long-term outcomes after very preterm birth, insight should be gained in the processes underlying the neurodevelopmental impairments in very preterm born children, with consideration of the heterogeneity in the population. This knowledge may ultimately aid the early identification of children that are particularly vulnerable for poor outcomes and the development of tailored interventions to improve these outcomes.

A method that can contribute to our understanding of brain functioning after very preterm birth and the potential identification of biomarkers for poor neurodevelopmental outcomes is electroencephalography (EEG). Brain oscillations in the low-frequency range involve the recruitment of large-scale networks, whereas high-frequency oscillations are involved in specific, focal cortical activation (Steriade et al., 1993). As children grow older, there is a typical decrease in low-frequency relative power accompanied by an increase in high-frequency relative power (Clarke et al., 2001, Uhlhaas et al., 2010). The increase of fast-wave activity is consistent with the reorganization of cortical networks during adolescence, which is characterized by a shift from diffuse to focal activation patterns (Durstun et al., 2006).

Few studies examined brain oscillations in individuals born (very) preterm or with (very) low birth weight beyond the neonatal period. These studies showed increased slow-wave resting-state activity in preterm/low birth weight individuals compared to controls (Rozhkova, 2008, Miskovic et al., 2009, Rommel et al., 2017). Findings with respect to fast-wave activity are mixed. One study reported increased fast-wave activity in preterm born 6.5–10-years-old children (Rozhkova, 2008), others showed no differences between preterm and full-term born adolescents aged 11–21 years (Rommel et al., 2017), while young adults born with extremely low birth weight exhibited decreased fast-wave activity compared to controls (Miskovic et al., 2009). Only one study linked oscillatory activity to functional outcomes and found that

increased delta power was associated with more ADHD symptoms in preterm born children (Rommel et al., 2017).

Cognitive processes are complex in nature and not manifested in a single frequency band, but instead associated with dynamic, distributed patterns of multiple oscillations (Başar et al., 2001, Kahana, 2006, Fan et al., 2007). The present study therefore examined patterns of brain oscillations across the different frequency bands in very preterm and full-term born adolescents to identify subgroups with different patterns of brain oscillations. EEG was measured during rest. It is well-established that the brain's intrinsic activity is essential to its function (Raichle and Snyder, 2007). In order to increase our understanding of the underlying neuronal mechanisms of neurodevelopmental impairments after very preterm birth, oscillatory activity patterns were related to broad indicators of functioning: intelligence, academic performance, and motor skills.

2. Methods

2.1. Participants

A Dutch cohort of 53 very preterm adolescents at 13 years of age was included in the present study. These adolescents were born in 2001–2003 and admitted to the level III neonatal intensive care unit of the Vrije Universiteit Medical Center in Amsterdam. Originally, 102 infants were included in a randomized placebo-controlled trial on the effects of enteral glutamine supplementation in the neonatal period. Further information about the intervention, its effects, and participant flow from birth to follow up at age 13 is reported elsewhere (Twilhaar et al., 2018b). A total of 61 adolescents participated in the follow-up at 13 years; 55 adolescents participated in all parts of the study and 6 adolescents and parents only filled in questionnaires. Two adolescents were not able to complete the electrophysiological recordings due to multiple disabilities and behavioral difficulties, resulting in a sample of 53 very preterm adolescents for the current study. Perinatal and demographic characteristics are shown in Table 1. The 61 controls were classmates of the very preterm participants or recruited from schools in the same geographical region from which the very preterm adolescents came from. Controls were born at term (≥ 37 weeks of gestation) and free of neurological, developmental, behavioral, or learning disorders.

Table 1
Demographic and perinatal sample characteristics.

	Very preterm ($n = 53$)	Full-term ($n = 61$)	p -value
Sex, n (%) boys	26 (49)	27 (44)	0.61
Age, years, M (SD)	13.31 (0.31)	13.27 (0.53)	0.62
Parental education, n (%) \geq bachelor degree or equivalent	33 (62)	38 (62)	1.00
GA, weeks, M (SD)	29.27 (1.54)		
BW, grams M (SD)	1253.34 (353.24)		
SGA ^a , n (%)	13 (25)		
Caesarean section, n (%)	29 (55)		
BPD ^b , n (%)	15 (28)		
IVH grade I/II, n (%)	8 (15)		
IVH grade III/IV, n (%)	1 (2)		
PVL, n (%)	2 (4)		
PDA, n (%)	7 (13)		
ROP, n (%)	4 (8)		
NEC, n (%)	0 (0)		
≥ 1 serious neonatal infection ^c , n (%)	35 (66)		
Glutamine supplementation, n (%)	26 (49)		

Note. M, mean; SD, standard deviation; GA, gestational age; BW, birth weight; SGA, small for gestational age; BPD, bronchopulmonary dysplasia; IVH, intraventricular haemorrhage; PVL, periventricular leukomalacia; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity; NEC, necrotising enterocolitis.

^a Birth weight < 10th percentile.

^b Oxygen requirement at 36 weeks postmenstrual age.

^c Sepsis, pneumonia, meningitis, pyelonephritis, or arthritis diagnosed based on a combination of clinical signs and positive culture.

2.2. Procedure

This study was conducted in accordance with the Declaration of Helsinki (2013) and approved by the local research ethics committee. Both parents and adolescents signed informed consent. EEG was recorded during 5 min eyes-open resting state.

2.3. Electrophysiological recordings and analysis

EEG was recorded at 2048 Hz from 64 electrodes using BioSemi ActiveTwo (BioSemi, Amsterdam, the Netherlands). Electrodes were placed according to the International 10–20 system. Electro-oculogram was registered with electrodes above and below the right eye, and at the external canthi. Reference electrodes were placed at the mastoids. Using Brain Vision Analyzer 2.1 (Brain Products, Munich, Germany) the data were filtered offline applying a 50 Hz notch filter and 0.1–40 Hz band-pass filter, and downsampled to 512 Hz. Channels were re-referenced to the average of the mastoids. Spherical spline interpolation was used to replace data for bad channels with a maximum of five channels. Ocular artifacts were estimated and corrected with a semi-automatic independent component analysis (Jung et al., 2000). Further artifacts were rejected based on the following criteria: maximum voltage step of 50 μ V, maximum absolute difference of 200 μ V, maximum peak-to-peak amplitude difference of 100 μ V, and minimum activity below 0.5 μ V. Fast-fourier transformation with a 10% Hanning window was applied to artifact-free segments of 3 seconds (≥ 30 segments) to calculate power (μ V²) in four frequency bands: delta (0.1–3.5 Hz), theta (4–7.5 Hz), alpha (8–13 Hz), and beta (14–30 Hz) (Schomer and Da Silva, 2012). Power was averaged across channels to calculate power at frontal (F3, Fz, F4), central (C3, Cz, C4), parietal (P3, Pz, P4), and occipital (O1, Oz, O2) sites. Relative band power was calculated by dividing absolute band power by total power in the 0.1–30 Hz range.

2.4. Intelligence, academic performance, and motor skills

Intelligence was assessed using the subtests vocabulary and block design of the Wechsler Intelligence Scale for Children, third edition (WISC-III) to estimate full-scale IQ. This short form has excellent reliability ($r = 0.91$) and correlates strongly ($r > 0.90$) with full-scale IQ (Sattler, 2008).

Standardized tests for reading comprehension (Weekers et al., 2011), spelling (Mols and Kamphuis, 2010), and arithmetic abilities (Janssen et al., 2010) developed by the Dutch National Institute for Educational Measurement, were used to assess academic performance. The tests are part of a nation-wide pupil monitoring system for grade 1–6 of primary school. The participant's most recent test scores—grade six test scores for most participants were used in the current study. Standard test scores for each academic domain were converted to z -scores. The average of z -scores for reading, spelling, and arithmetic was used as a measure of general academic performance.

Movement Assessment Battery for Children, second edition (MABC-II) measures motor skills in three domains: manual dexterity, ball skills, and static and dynamic balance. Raw total scores were converted to z -scores and used as outcome measure in the analyses.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 24 (IBM SPSS Statistics, NY, USA) and Mplus 7.31 (Muthén & Muthén, 1998–2015). Winsorizing was applied to deal with extreme outliers (values more than three times the interquartile range below the first and above the third quartile) (Howell, 2009). Absolute and relative

power data were log-transformed to normalize skewed distributions. Group differences in age, sex, and parental education level were assessed using t - and η^2 -tests. Log-transformed absolute and relative delta, theta, alpha, and beta power were analyzed using mixed-effects ANOVAs with group (very preterm, full-term) as between-subjects factor and electrode site (frontal, central, parietal, occipital) as within-subjects factor. Age and sex were included as covariates. Benchmarks for partial η^2 were 0.01 (small), 0.06 (medium), and 0.14 (large) (Cohen, 1988). The Benjamini-Hochberg procedure was applied for false discovery rate (FDR) control ($q = 0.05$) to account for multiple comparisons.

Latent class analyses (LCA) were performed to examine whether subgroups of adolescents can be defined based on the observed relative power in the delta, theta, alpha, and beta frequency band. Information criteria (Bayesian Information Criterion [BIC] and adjusted BIC) and likelihood-based indexes (Lo-Mendell-Rubin [LMR] and bootstrap likelihood ratio test [BLRT]) were used to decide on the number of classes (Nylund et al., 2007). The BIC and aBIC are log-likelihood-based fit indices that apply a penalty based on the number of parameters and sample size. The sample size penalty is reduced in the aBIC. Smaller values represent better model fit. With the LMR and BLRT, the improvement in fit for a model with k classes compared to a model with $k-1$ classes was tested, with a p -value < 0.05 indicating significantly improved fit with inclusion of one class more. Subsequently, class membership was related to neurodevelopmental outcomes using the BCH method in Mplus as proposed by Vermunt (2010) which uses weights based on the measurement error of the latent class variable to estimate the mean of a distal outcome. Wald χ^2 was used to test the equality of means across classes. Lastly, birth status (very preterm, full-term) was used to predict class membership by means of the R3STEP command in Mplus, which corrects for classification error (Vermunt, 2010).

3. Results

3.1. Data inspection

The average number of artifact-free segments was lower in the very preterm group ($M = 76.16$, $SD = 18.21$) than the full-term group ($M = 85.29$, $SD = 13.76$; $t(1, 112) = 2.92$, $p = 0.004$). Absolute delta and beta power were maximal in (pre)frontal regions, theta power was maximal at frontocentral regions, and alpha power was maximal at occipital sites in both groups (Fig. 1).

3.2. Group differences

There were no differences between very preterm children in the glutamine-supplemented and placebo group for EEG band power (eTable 1). Mean absolute and relative power for very preterm and full-term adolescents for each frequency band is presented in Table 2. Very preterm born adolescents exhibited significantly lower relative beta power than full-term born adolescents. Moreover, there was a significant group*site interaction for absolute alpha power. Contrast analyses revealed that the difference in alpha power between parietal sites and other sites was smaller in preterm adolescents than controls (occipital: $F(1, 112) = 7.64$, $p = 0.007$; central: $F(1, 112) = 8.73$, $p = 0.004$; frontal: $F(1, 112) = 11.04$, $p = 0.001$). Absolute delta power was increased in very preterm born adolescents compared to controls, but this finding was non-significant after FDR control.

3.3. Patterns of brain oscillations

To identify subgroups of adolescents with different activity patterns, model with an increasing number of classes were fitted

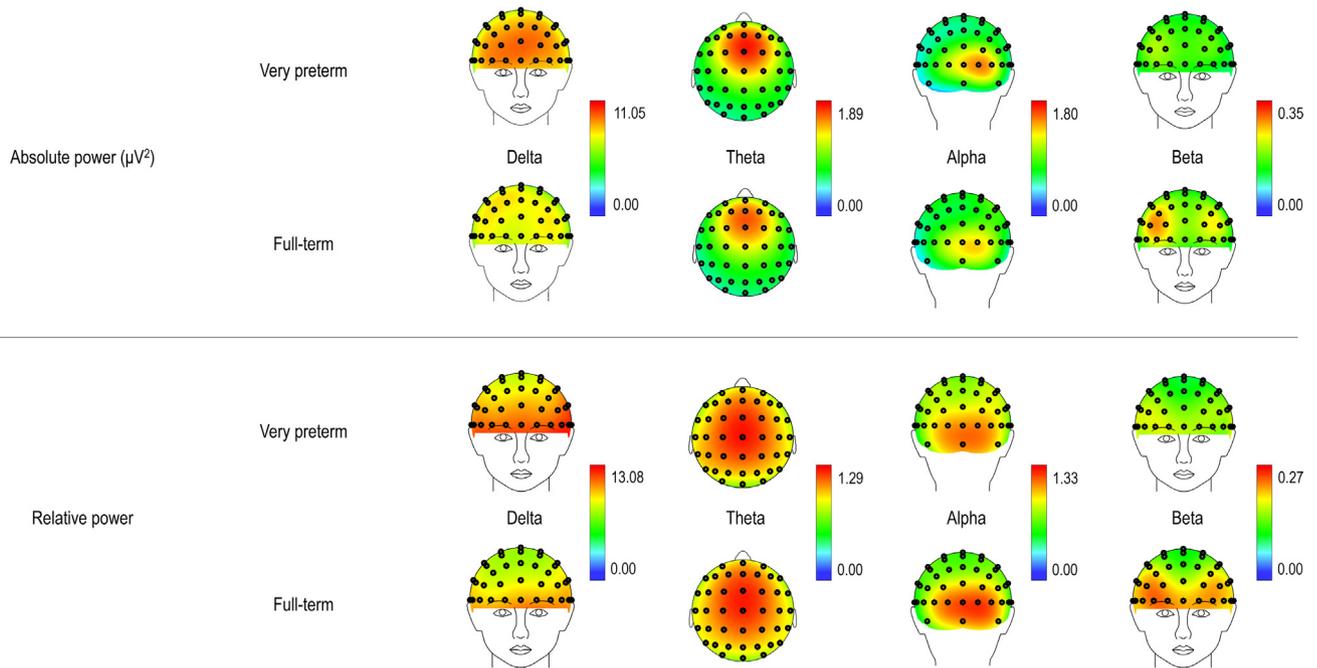


Fig. 1. Topographies of the total absolute and relative power in each frequency band during eyes-open resting state in the very preterm and full-term born sample.

using LCA. Model fit statistics are presented in Table 3. A 4-class solution was favored based on these statistics. The entropy (0.91) reflects clear class identification and class membership probabilities were high (0.92–0.99). The two largest classes (class 1 and 2) differentiate between adolescents with higher relative delta and lower theta, alpha, and beta power in class 1 compared to adolescents with most likely membership of class 2. Class 1 was further differentiated by a small group of individuals (class 4) with a similar pattern compared to class 1 but significantly higher delta and lower theta, alpha, and beta power (see Fig. 2). Similarly, class 3

identified a small number of adolescents with a similar activity pattern as class 2 but with lower delta, and higher theta and alpha power relative to class 2.

As a next step, class membership was related to three distal outcomes: intelligence, academic performance, and motor functioning. Fig. 3 presents the estimated means of these outcomes for each class. Adolescents with most likely membership of class 4 had significantly lower estimates of IQ than adolescents most likely assigned to class 3 ($\chi^2 = 10.87, p = 0.001, d = 1.49, 95\% \text{ CI } 0.44, 2.41$), class 2 ($\chi^2 = 14.15, p < 0.001, d = 1.31, 95\% \text{ CI } 0.56,$

Table 2

Mean EEG band power (absolute and relative) of very preterm and full-term born adolescents and statistics of group and group-by-site interaction effects for eyes-open resting state and during task performance.

			Very preterm	Full-term	F	p	η_p^2
Delta	Absolute	Group	0.82 (0.13)	0.77 (0.13)	5.33	0.02	0.05
	Relative	Group	1.89 (0.05)	1.87 (0.05)	2.23	0.14	0.02
Theta	Absolute	Group	-0.35 (0.16)	-0.35 (0.16)	0.04	0.85	0.00
	Relative	Group	0.74 (0.15)	0.78 (0.15)	3.03	0.08	0.03
Alpha	Absolute	Group*Site			3.59	0.01	0.03
	Relative	Group	0.79 (0.28)	0.83 (0.28)	0.65	0.42	0.01
Beta	Absolute	Group	-1.23 (0.20)	-1.16 (0.20)	2.89	0.09	0.03
	Relative	Group	0.51 (0.18)	0.61 (0.18)	9.68	0.002*	0.08

Note. Statistics of the group*site interaction were only reported if this effect was significant. If not, mean power averaged over sites was reported for each group together with associated statistics for the main effect of group.

* Significant at a false discovery rate criterion of 0.05.

Table 3

Model fit statistics.

Classes	Loglikelihood	Entropy	BIC	aBIC	LMR, p-value ^a	BLRT, p-value ^a
1	233.08		-425.26	-453.55		
2	303.73	0.83	-545.89	-586.98	0.05	<0.001
3	344.83	0.89	-604.40	-661.29	0.13	<0.001
4	372.48	0.91	-636.03	-708.72	0.32	<0.001

^a p < 0.05 indicates improved fit compared to a solution with one class less.

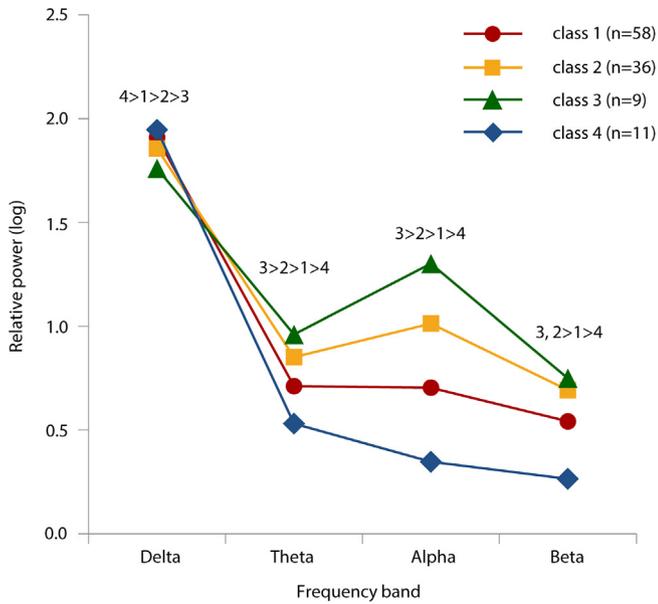


Fig. 2. Patterns of relative delta, theta, alpha, and beta power for each class during eyes-open resting state.

2.01), and class 1 ($\chi^2 = 8.72, p = 0.003, d = 0.87, 95\% \text{ CI } 0.20, 1.52$). Similarly, estimates for academic performance were lower for class 4 compared to class 3 ($\chi^2 = 4.38, p = 0.04, d = 0.91, 95\% \text{ CI } 0.05, 1.90$), class 2 ($\chi^2 = 7.58, p = 0.006, d = 0.98, 95\% \text{ CI } 0.26, 1.67$), and class 1 ($\chi^2 = 5.61, p = 0.02, d = 0.81, 95\% \text{ CI } 0.15, 1.47$). Estimates of motor functioning were significantly lower for adolescents with most likely membership of class 4 compared to class 3 ($\chi^2 = 4.07, p = 0.04, d = 0.85, 95\% \text{ CI } 0.10, 1.93$) and class 2 ($\chi^2 = 5.87, p = 0.02, d = 1.28, 95\% \text{ CI } 0.54, 1.98$), but not to class 1.

Lastly, class membership was predicted by birth status. Very preterm born adolescents were more likely than full-term controls to be assigned to class 4 compared to the other classes (class 3: OR = 10.61, 95% CI 0.64, 20.58, $p = 0.04$; class 2: OR = 8.38, 95% CI 1.35, 15.41, $p = 0.03$; class 1: OR = 6.10, 95% CI -0.86, 13.06, $p = 0.07$).

4. Discussion

The present study revealed different patterns of brain oscillations in very preterm born adolescents compared to full-term con-

trols. Differences were largest for the beta frequency band, with very preterm born adolescents exhibiting decreased power relative to controls during resting state. Four subgroups were distinguished with different distributions of relative delta, theta, alpha, and beta power. A subgroup exhibiting higher delta and lower theta, alpha, and beta activity compared to the other three subgroups was characterized by poorer intelligence, academic performance, and motor abilities. Moreover, very preterm born adolescents were more likely than full-term born adolescents to be members of this subgroup.

The alterations in oscillatory activity during adolescence indicate that disruption of neonatal brain development as a consequence of very preterm birth and associated pathogenic factors has long-lasting effects on later brain maturation. These alterations were most apparent in the beta frequency band. Brain maturation during adolescence is characterized by a decrease in low-frequency relative power, an increase in high-frequency relative power, and a decrease in absolute power in all frequency bands (Clarke et al., 2001, Uhlhaas et al., 2010). Structural changes that are thought to give rise to these neurophysiological changes are synaptic pruning and myelination (Segalowitz et al., 2010). These structural changes are associated with improved neurocognitive function (Blakemore and Choudhury, 2006). The lower relative beta power shown in very preterm born adolescents compared to controls could indicate a delay of normal maturational processes during adolescence or persisting brain alterations. Similar findings of Miskovic et al. (2009), showing decreased beta activity in extremely low birth weight adults, may suggest the latter. However, longitudinal evaluations of oscillatory patterns combined with MRI are needed to gain insight in the structural abnormalities and maturational processes that give rise to altered oscillatory activity after very preterm birth as shown in the present study.

Few studies addressed the large individual variation in outcomes among very preterm born children. However, in order to early identify those very preterm born children that have an increased risk for poor outcomes, it is crucial to understand the specific mechanisms underpinning impaired versus relatively unimpaired functioning after very preterm birth. The present study is the first to account for individual variation in neurophysiological activity among very preterm born adolescents. A subgroup of adolescents with higher delta activity and lower theta, alpha, and beta activity (“class 4”) compared to the other subgroups tended to show poorer intelligence, academic performance, and motor abilities than the other subgroups. Importantly, very preterm born adolescents were more likely than controls to show this activity

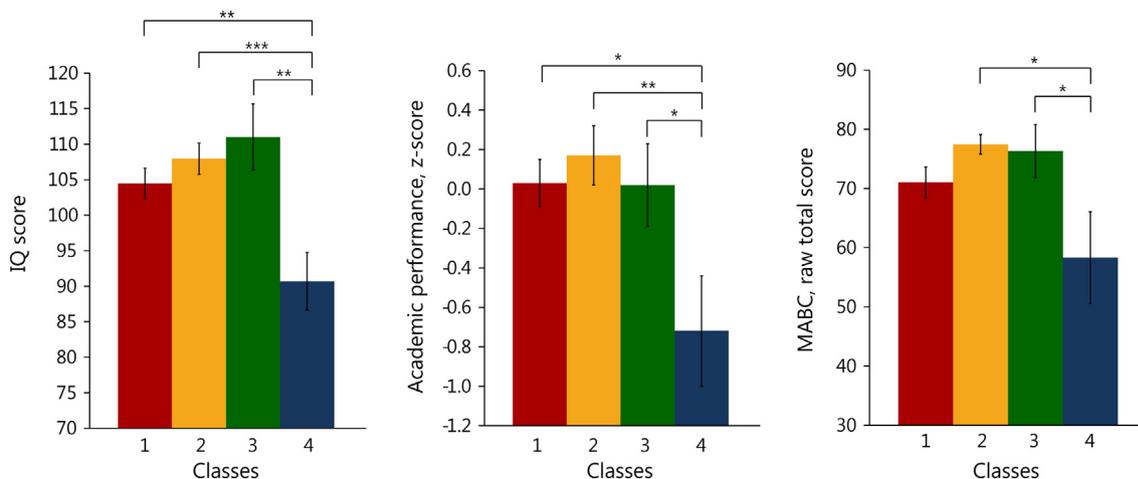


Fig. 3. Estimated means of IQ, academic performance, and motor skills per class. IQ, intelligence quotient; MABC, Motor Assessment Battery for Children. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

pattern. This suggests that oscillatory activity patterns deviating most from patterns that characterize normal maturation during adolescence (i.e. a decrease in low-frequency and an increase in high-frequency power) are associated with impaired functional outcomes. Although this pattern resembled activity patterns in younger children, again, longitudinal research is necessary to determine whether this indicates delayed maturation or persisting brain alterations. Moreover, future studies should focus on perinatal and demographic factors to further characterize subgroups of very preterm born children at increased risk for poor functional outcomes.

The identification of subgroups in the current study and subsequent associations with functional outcomes and very preterm birth were limited by the relatively small sample size. The results should therefore be interpreted with caution and await replication in larger samples. Although the size of “class 4” was small, which affected the precision of estimates of functional outcomes for this class, its consistent associations with significantly poorer outcomes and very preterm birth supports the consideration of this class as a meaningful subgroup of individuals. The association of brain oscillations with both functional outcomes and very preterm birth as shown in the present study is promising because it implicates that specific oscillatory patterns may serve as biomarkers for poor functional outcomes in this population. The search for electrophysiological biomarkers is important as such biomarkers could make a valuable contribution to the early identification of very preterm children at risk for poor outcomes given that EEG is a non-invasive affordable technique that can be easily implemented in clinical practice. The present findings demonstrate that not activity in a single frequency band but particularly the combination of oscillations with different frequencies may provide important insight in the underlying mechanisms of neurodevelopmental impairment after very preterm birth. Future studies should investigate whether the same approach could differentiate between subgroups in infancy and if so, whether oscillatory patterns during infancy are related to functional outcomes later in life.

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Declaration of Competing Interest

Prof. dr. R.M. van Elburg is an employee at Danone Nutricia Research, Utrecht, the Netherlands. The other authors have no conflicts of interest to disclose.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clinph.2019.05.002>.

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