Abstract

Background: Thyroid hormones are indispensable for brain development. Neonates born at an extremely low gestational age (ELGAN) often suffer from transient hypothyroxinemia of prematurity (THOP). The effect of this condition on the early brain development is a controversial and much disputed topic. Automated analysis of the EEG is an objective method to assess brain maturation in preterm infants. We hypothesized that in case of low circulating thyroid hormone levels, brain maturation is affected, which will be reflected by disturbances in EEG.

Methods: In this retrospective single-center study, circulating fT4 levels on day 0 (time 1) and at the end of the first week of life (time 2) were collected and delta (Δ) fT4 (=$fT4_{time2}$-$fT4_{time1}$) was calculated in 63 ELGANs. Brain maturation was quantified by complexity and spectral features of the EEG around term age. Relevant clinical data were collected. Linear regression models are used to investigate the relationship between the change in fT4 level and the brain maturation assessed via EEG features at TEA.

Results: We could not identify a significant association between the thyroid hormone levels and the maturational EEG features.

Conclusion: No significant relationship between temporary low thyroid hormone levels in preterm infants in the first week of life and maturational EEG features was found. However, the limited sample size and the retrospective study design are important limitations, therefore prospective studies are recommended.

Keywords: EEG; THOP; preterm neonate

1. Introduction

Thyroid hormones (THs) are important developmental hormones in different kind of species [1]. In humans, they play a vital role in fetal and neonatal developmental processes in general, and in particular in the developing brain [2,3]. This is illustrated in Figure 1. By activation of numerous thyroid responsive genes, they regulate several neurodevelopmental processes, such as neurogenesis, myelination, dendrite proliferation and synapse formation [4]. These actions occur in specific time windows and are initiated by binding of the active hormone T3 to nuclear receptors encoded by the thyroid hormone receptor genes (THRA, THRB). Most T3 is produced by deiodination of T4 in peripheral tissues [5]. Also in the human fetus, cerebral T3 availability is primarily generated by local T4 deiodination [6]. Fetal TH metabolism starts progressively from mid-gestation. Up to that point, the fetus is completely dependent of trans-placental maternal TH supply, which remains present until birth. Only then, the infant's TH system acts completely autonomously [7]. However, in preterm birth, the trans-placental maternal TH supply is suddenly interrupted while the infant's thyroid system is still immature. These elements are contributing to the development of transient hypothyroxinemia of prematurity (THOP). THOP is characterized by low circulating total and free T4 concentrations, without the expected increase in pituitary thyroid-stimulating hormone (TSH) secretion. This phenomenon is more severe in patients born at lower gestational age (GA) [7]. For more than three decades, THOP has been a debatable research topic. Heterogeneity in THOP definitions, difficulties with TH assessment, identifying patients at risk and a lack of sufficiently powered studies add to the controversy [8]. Recently, we developed a new approach to THOP by studying trends in circulating TH levels in the first week of life. We calculated the difference between TH levels at the end of the first week of life (time 2) and cord blood (time 1), the so called Δ and found that immaturity was the most important contributing factor to a negative Δ [9].

Since THs play a critical role in the early development of the brain, we expect alterations in the brain maturation of ELGANs with THOP. The EEG is the most common tool for continuous bedside monitoring of the cerebral function of infants in the neonatal intensive care unit. The fast brain

development during the first weeks after birth is reflected in a continuously changing EEG. Automated analysis of these rapidly changing EEG patterns can be used to quantify the brain maturation in order to monitor the brain development and to predict the neurodevelopmental outcome of the infant. Various characteristics of the neonatal EEG have been investigated, such as the continuity of the EEG [10,11] the (relative) power in specific frequency bands [12–14], the spatial organization of the EEG [15,16] and its complexity [17]. To the best of our knowledge, the impact of THOP on brain maturation quantified using EEG features has never been assessed before. Nevertheless, diffuse slowing of background activity on EEGs in adults with hypothyroidism has been demonstrated [18]. In paediatric patients with congenital hypothyroidism, altered sleep state organization on polysomnographic studies at toddler age has been shown [19]. Finally, in a chicken-model of brain development, mild hypothyroidism was associated with a delay in developmental changes in basal EEG patterns [20]. Therefore, we hypothesized that temporary low circulating fT4 levels in preterm infants on a critical moment of their brain development might affect brain maturation, which might be reflected by alterations in electro-cortical maturation on the EEG.

Previous research has established that spectral features and the complexity of the EEG are powerful characteristics to track brain maturation in preterm infants [13,17]. Therefore, we decided to assess the brain maturation in this study by means of EEG complexity and frequency domain features. Features related to the continuity of the EEG were considered less appropriate since the EEG is recorded around term equivalent age.

The aim of this study was to investigate whether a delayed brain maturation could be observed in ELGANs with a lower TH levels in the first week of life.

Materials and Methods

2.1. Database

Patient selection

In this single-center retrospective study, quantitative EEG-maturation at (near) term age (PMA: 35 weeks 2 days - 46 weeks 5 days) in 65 preterm infants born before 28 weeks of gestation (GA: 23 weeks and 6 days - 27 weeks 5 days), was analysed in relation to the evolution of their fT4 levels in the first week of life. The EEG signals were collected in the framework of the NeoGuard [21] and Resilience study (June 2012 - May 2017) at University Hospitals Leuven. Relevant clinical data were collected: gestational age (GA), birth weight, presence of intrauterine growth restriction (IUGR), CRIB score, moderate or severe bronchopulmonary dysplasia (BPD, need for supplemental oxygen/ventilation at 36 weeks GA), retinopathy of prematurity (ROP) with need for laser therapy, persistent ductus arteriosus (PDA), necrotizing enterocolitis (NEC, defined as Bell's stage II & III), sepsis with positive blood culture, cranial ultrasound abnormalities (grade II-IV IVH and localized infarct), levothyroxine treatment after the first week of life. TH supplementation (levothyroxine 10 µg/kg) for 14 days was initiated when fT4 levels were below 0.8 ng/dL at the end of the first week of life, according to the treatment regimen of the unit. Parental informed consent was obtained. The study was approved by the local ethical committee of the University Hospitals Leuven (S61028).

EEG monitoring

All infants had an overnight polysomnography recording with multichannel EEG, electrocardiogram, oxygen saturation, chin electromyogram, 2 electrooculograms, piezo-electric belts to measure abdominal and thoracic respiratory effort, and a nasal thermistor for airflow monitoring at a PMA between 35 weeks 2 days and 46 weeks 5 days.. The EEG recordings were measured using 9 electrodes: Fp1, Fp2, C3, C4, T3, T4, O1, O2 and reference Cz, placed according to the international 10-20 EEG recording system (BrainRT, OSG equipment, Mechelen, Belgium). A monopolar EEG setup was used and the reference electrode Cz is not considered during the analysis. The EEG time series were sampled at 250 or 256 Hz. The duration of the EEG recording is in the range from 3 h 27 min to 15 h 24 min. The

average recording length is equal to 9 h 55 min with a standard deviation of 1 h 15 min, which is sufficient to cover all sleep stages. No visual preselection of the data was performed.

2.2. Thyroid hormone function

As part of the clinical standard care protocol, fT4 levels on the first day of life (preferably cord blood) and at the end of the first week of life were determined by competitive immunoassay with ECL (ElectroChemiLuminescence), Hitachi/Roche-Modular E. Blood samples were primarily taken through an arterial line, or by vena puncture, when no arterial line was available. The evolution of the fT4 levels through the first week of life was calculated as:

$$\Delta fT4 = fT4 \text{ (at the end of the first week of life)} - fT4 \text{ (at the day of birth)}.$$

Statistical analysis

Statistical analyses were done using IBM SPSS Statistics. Shapiro-Wilk was used for normality testing. Normally distributed data were analysed using the independent-samples t-test. The nonparametric Mann-Whitney U test was used when data were not normally distributed. Chi-square test for independence and Fisher's exact test were used for categorical variables. Data are expressed as mean +/- SD or median with interquartile range. Statistical significance was assumed for a two-sided p-value < 0.05.

2.3. Automated EEG analysis

Preprocessing

The first step in the automated analysis of EEG is preprocessing of the data. The EEG time series is bandpass filtered between 0.5 and 40 Hz in order to remove low and high frequency noise. In addition, a notch filter at 50 Hz is applied to avoid distortion by powerline interference.

After filtering the EEG, the signal is down-sampled with a factor two in order to reduce the computational complexity during further analysis.

Afterwards, an automated artefact detection step is used to identify the segments in the filtered EEG which could contain artefacts. For this automated artefact detection, the EEG is segmented into non-

overlapping windows of 100 s. If more than 5% of an EEG segment consists of missing values or has an absolute value of the amplitude higher than 150 μV, the segment is annotated as potential artefact and is not taken into account in further analysis. In addition, segments with a standard deviation above 50 μV or below 0.001 μV are discarded.

This analysis is performed per channel and if an artefact is identified on more than half of the EEG channels, the segment is excluded from all channels.

At last, the complete recording is considered to be of poor quality if for more than half of the channels (> 4) more than 50% of the segments are indicated as potential artefact. Because of this, two recordings were excluded from the analysis.

Feature extraction

After preprocessing the EEG time series, the maturational features are extracted from the EEG. On the one hand, spectral features are computed, on the other hand the complexity of the EEG is assessed.

Spectral features

In order to compute the spectral features, the power spectral density of each non-overlapping 25 s EEG segment is estimated. Welch's method was adopted using a 4 s Hamming windows with 50 % overlap. The relative power in the delta 1 (δ_1: 0.5 - 1 Hz), δ_2: 1 - 4 Hz), theta (θ: 4 - 8 Hz), alpha (α: 8 - 12 Hz) and beta (β: 12 - 30 Hz) band is then computed by integrating the modified periodograms in these frequency bands and dividing by the total power in the band from 0.5 to 30 Hz. In addition to the relative bandpower, the spectral edge frequency (SEF) 75 and 90 are also computed. This corresponds to the frequency below which respectively 75% and 90% of the total power of the signal is located. In total 7 spectral features are extracted from each clean 25 s EEG segment, resulting in 56 (7 features x 8 channels) features per multichannel EEG segment. The average of each feature across all EEG segments within the recording is then computed, so that one value for each feature is obtained per EEG recording.

Complexity features

The complexity of each clean multichannel EEG segment of 100 s is assessed by computing the multiscale entropy. Multiscale entropy is a method to evaluate the complexity of a time series by assessing the regularity of the signal across a range of temporal scales [22]. Its computation is composed of two steps. First, the EEG is coarse grained to represent the system dynamics at multiple scales. These coarse grained realizations are computed by averaging the data points within a non-overlapping window with its length equal to the scale factor τ. So, the coarse grained time series at scale τ is obtained as:

$$y_j^\tau = \frac{1}{\tau} \sum_{i=(j-1)\tau+1}^{j\tau} x_i \,, \qquad 1 \leq j \leq \frac{N}{\tau}.$$

Second, the sample entropy of each coarse grained time series is computed. Sample entropy is a measure of the regularity or predictability of a time series. It is computed as the negative natural logarithm of the conditional probability that sequences of m consecutive data points matching within a tolerance r, will also be similar when an extra data point (m+1) is added to the sequence [23]. Two patterns are considered similar if they match within a tolerance r. So, the formula of sample entropy for a time series with length N, a tolerance r and a pattern length m can be written as:

$$SampEn(m, r, N) = -ln\frac{A}{B}$$

where B denotes the number of pattern matches at length m and A denotes the number of matches at length m+1.

In this study, the template length m is set equal to 2 and the tolerance r is defined as 0.2 times the standard deviation of the time series. The multiscale entropy is computed for scales from 1 up to 20, so in total 20 sample entropy values will be computed for each channel of a 100s EEG segment. Plotting these sample entropy values in function of the scale factor τ results in a multiscale entropy curve, showing the entropy of the signal at multiple scales. Three features are extracted from the multiscale entropy curve. First of all, the complexity index (CI), estimated by computing the area under the multiscale entropy curve [24–26]. This feature provides insight in the regularity of the signal over all

considered scales. Second, the average slope of the multiscale entropy in the small scales, τ: 1 - 5, is computed. The last feature is the maximum value of the multiscale entropy curve.

Thus, for each multichannel EEG segment three complexity features are extracted, resulting in 24 values for each 100s segment (three for each EEG channel). Similarly to the frequency domain features, each of the complexity features is averaged among all 100 s segments within the EEG recording. Therefore, each EEG recording is characterized by 24 complexity features.

Regression analysis

A regression analysis is performed in order to investigate whether poor thyroid function is related to abnormal brain maturation reflected in EEG features around term equivalent age. First, the relationship between the delta fT4 and each of the maturational EEG features is investigated per channel. As the EEG recordings took place at different postmenstrual ages and an increase/decrease of the extracted maturational features with PMA is expected, this might lead to biased results. To account for this confounding variable, the postmenstrual age is included in the multiple regression model. Moreover, only a subset of the cohort was treated with levothyroxine and some of the neonates suffered from an intraventricular haemorrhage (IVH) as presented in Table 1. Both factors are expected to influence the subsequent brain development, and as a consequence also the appearance of the EEG around term equivalent age. That's why these variables are included as additional independent variables.

The independent variables can be represented in a matrix $\mathbf{X} \in \mathbb{R}^{N\,x\,d}$, with N the number of observations, corresponding to 63 EEG recordings, and d = 4 representing the number of independent variables (delta fT4, postmenstrual age, treatment and IVH). Note that ΔfT4 and postmenstrual age are continuous variables, while treatment and IVH are dummy variables (either 0 or 1). Eventually, for each channel a multiple linear regression model is fitted between the independent variables and each of the maturational EEG features. Then, the significance of the regression coefficient of interest, the one indicating the relationship between ΔfT4 and the EEG feature holding all confounding variables constant, was tested. The significance level was set at 0.05.

While the multiple regression model described above can indicate which features are significantly related to the fT4 level evolution for a particular EEG channel, it suffers from the multiple comparisons problem as the significance for each feature for all 8 channels is tested separately [27]. In order to reduce the risk of incorrectly rejecting the null hypothesis, we have also developed a regression model in which all EEG channels are considered simultaneously. Since the different observations are no longer independent (8 channels from the same neonate), a mixed-effects model with a random intercept using the patient as grouping variable is adopted. Moreover, the EEG channel is included as additional independent variable, because channel-dependent differences are expected relying on the fact that not all brain regions develop equally fast. This will result in a mixed-effects regression model investigating the relationship between the change in delta fT4 and each EEG maturational feature considering 504 observations (63 EEG recordings x 8 channels). In the same way as the channel-wise analysis, the significance of the regression coefficient reflecting the association between ΔfT4 and the EEG feature is tested.

3. Results

3.1. Thyroid hormone function

Both EEG recordings at term equivalent age and fT4 levels at birth and during the first week of life were available in 65 ELGANs, but 2 patients were removed from the analysis during the pre-processing phase because of poor EEG quality. 27 (43%) neonates had a positive ΔfT4 level in the first week of life, whereas 36 (57%) neonates had a negative ΔfT4 level. Patient characteristics are summarized in Table 1. Gestational age was significantly lower in the negative ΔfT4 group ($p < 0.01$), (Figure 2a). Moreover, infants with negative ΔfT4 had a significant higher rate of supplementation therapy with levothyroxine ($p < 0.01$). Although the number of patients with intracranial ultrasound abnormalities were not significantly different between both groups, we included IVH as a confounding factor in the analysis since these brain lesions can theoretically also affect the EEG appearance. Figure 2b shows the fT4 levels at day of birth, at the end of the first week of life and the change between the measurements in patients with a positive and a negative ΔfT4. It can be seen that the difference in fT4 level at the end of the first week of life and the change in fT4 is significantly different between the group with positive ΔfT4 and negative ΔfT4.

3.2. Automated EEG analysis

Regression analysis was used to examine whether thyroid hormone evolution during the first week of life is associated with maturational features extracted from the EEG. First, this relationship is evaluated for each EEG channel separately. Only for one of the maturational EEG features, more specifically the spectral edge frequency 75 in EEG channel T4, a significant inverse relationship with the delta fT4 was found. Second, a mixed-effects model, fitting the relationship between delta fT4 and a specific maturational EEG feature (including all channels), was also considered. The results of this analysis are presented in Table 2. As can be seen from the table, none of the maturational EEG features are significantly related to the change in free thyroxine concentrations during the first week of life ($p > 0.05$).

4. Discussion

In this retrospective, observational study, the relationship between changes in fT4 hormone during the first week of life and a set of maturational features extracted from the EEG is explored by means of a regression analysis. Only for the relationship between the delta fT4 and the spectral edge frequency 75 in the T4 channel a significant inverse association was observed. However, the p-value was close to significance level (p=0.048) and due to the many statistical tests, it is likely that this corresponds to a false positive. Actually, we would even expect a positive association between ΔfT4 and SEF, given that the dominant frequency of the EEG increases with ageing. Generally, the multiple linear regression model and mixed effects model, corrected for possible confounding by postmenstrual age, treatment or IVH, did not demonstrate a significant association between disturbed thyroid function and EEG features at term age.

As far as we know, this is the first study that investigates THOP in relation to functional brain maturation assessed via EEG. In contrast to our study, De Vries et al. described a relationship between hypothyroxinemia and neural maturation in very low birth weight preterm infants [28]. They demonstrated that low thyroxine concentrations are associated with a delay in progression of peripheral nerve conduction velocity in preterm infants at term equivalent age, possibly due to decreased central and peripheral myelin formation.

In the TIPIT-trial, a randomized placebo-controlled trial investigating the role of prophylactic levothyroxine therapy in ELGANs, no advantage of prophylactic supplementation therapy could be demonstrated [29]. However, in a sub-study with MRI, the lowest fT4 levels were associated with markers of poorly organized brain microstructure [30]. The association between EEG and MRI findings in very preterm infants has already been demonstrated and contributed to the outcome prediction at 24 months [31]. It would therefore be of further interest to study THOP in relation to both EEG and MRI findings and in relation to the long term neurodevelopmental outcome.

Several limitations of the current study need to be considered. First of all, this was a retrospective study. Although fT4 levels at day 0 were usually obtained in cord blood, to exclude the effect of the

TH surge during the first hours of life [7], there is uncertainty whether this was the case in all infants. Albeit the TH surge in ELGANs is usually limited compared to term infants [7], increased TH levels in the first hours of life may have influenced the ΔfT4 results. An additional issue is related to the equipment used to measure the free thyroxine concentrations. Commercial kits, as used in this study, are known to underestimate the fT4 levels when there is low TH protein binding capacity. The golden standard for fT4 measurement is the expensive and labour-intensive equilibrium dialysis, nevertheless this is rarely used in the clinical setting. With this technique, depression of T4 levels and maintenance of fT4 levels at 2 weeks of age in ELGANs were described [32]. Information about whether blood samples were collected from an arterial line or by vena puncture was also not available. Heparin is used for patency of the arterial line. Heparin releases plasma lipases, leading to increased plasma free fatty acids. These compete with T4 for binding to plasma binding proteins, causing an increase in fT4 levels [33]. Therefore, these alterations might have influenced the obtained ΔfT4. Besides, there are important differences in patient characteristics between the groups with negative and positive ΔfT4 levels. The group with negative ΔfT4 levels had a significantly lower GA. This is in accordance with the fact that immaturity is an important contributing factor to the development of THOP [34]. Although the infants in the negative ΔfT4 group were significantly younger than the infants in the positive ΔfT4 group, their CRIB scores, reflecting the severity of disease, were comparable. Moreover, as illustrated in Figure 3, the EEG signals are generally recorded at a higher age for neonates with a disturbed thyroid function. EEG recordings at a higher PMA are expected to reflect increased brain maturation. As a result, patients with a negative ΔfT4 evolution are biased towards better maturational EEG features. Therefore, correction for the postmenstrual age was deemed necessary. Although current evidence for THOP treatment is lacking, as is the case in several topics in neonatal medicine [35], infants with the lowest fT4 levels received levothyroxine treatment. As this might affect the patterns observed in the EEG, this is also considered as a confounding variable in the regression analysis. The current investigation was also limited by the lack of information on the sleep-wake cycling during the EEG recording. In this study the EEG complexity of the complete recording was considered, however it is generally known that the EEG

complexity is dependent on the sleep state of the infant [17]. Hence, a different proportion of the sleep might have affected the average value of the maturational EEG feature. An additional uncontrolled factor is that part of the artefacts might not have been removed from the EEG during the preprocessing phase and influenced the subsequent analysis. Finally, the regression models used in this study assumed a linear relationship between the ΔfT4 levels and metrics to quantify brain maturation extracted from the EEG. However, the evolution of the maturational EEG features have been extensively studied up to around 40 weeks PMA, but little is known about the trends beyond term equivalent age. Therefore, it is suggested that further research investigates thyroid function in relation to functional brain maturation assessed at the same postmenstrual age.

Despite these limitations, this study is the first step towards a new, neurophysiological approach to obtain a better understanding of THOP. As many of the aforementioned limitations are related to the dataset, further prospective studies accounting for these weaknesses are recommended. These prospective studies exploring the impact of THOP on EEG maturation, in relation to the neurodevelopmental outcome are required to improve insights in the development and impact of THOP.

5. Conclusion

This study was set out to determine the relationship between the evolution of free thyroxine levels during the first week of life and functional brain maturation assessed via EEG features at term equivalent age. For this purpose, linear regression analyses accounting for possible confounding by PMA, levothyroxine treatment and IVH has been performed. No significant correlations between thyroid function and maturational EEG features were found.

Research Funding

This research was funded by the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ERC Advanced Grant: BIOTENSORS (n°339804) and Bijzonder Onderzoeksfonds KULeuven (BOF): The effect of perinatal stress on the later outcome in preterm babies (# C24/15/036). Mario Lavanga is a SB PhD fellow at Fonds voor Wetenschappelijk Onderzoek-Vlaanderen (FWO), supported by Flemish government. AE has a clinical research mandate of the University Hospitals Leuven (KOOR-mandate).

References

1. Laudet, V. The Origins and Evolution of Vertebrate Metamorphosis. *Curr. Biol.* **21**, R726–R737 (2011).
2. De Groot, L. J. *et al.* Thyroid Hormones in Brain Development and Function--Endotext. in *Endotext* (MDText. com, Inc., 2000).
3. Forhead, A. J. & Fowden, A. L. Thyroid hormones in fetal growth and prepartum maturation. *J. Endocrinol.* **221**, R87--R103 (2014).
4. Bernal, J. Thyroid hormone receptors in brain development and function. *Nat. Clin. Pract. Endocrinol. Metab.* **3**, 249–259 (2007).
5. Visser, T. J. Thyroid hormone transport across the placenta. *Ann. Endocrinol. (Paris).* **77**, 680–683 (2016).
6. Kester, M. H. A. *et al.* Iodothyronine Levels in the Human Developing Brain: Major Regulatory Roles of Iodothyronine Deiodinases in Different Areas. *J. Clin. Endocrinol. Metab.* **89**, 3117–3128 (2004).
7. Fisher, D. A. Thyroid System Immaturities in Very Low Birth Weight Premature Infants. *Semin. Perinatol.* **32**, 387–397 (2008).
8. Eerdekens, A. *et al.* Review shows that thyroid hormone substitution could benefit transient hypothyroxinaemia of prematurity but treatment strategies need to be clarified. *Acta Paediatr.* **108**, 792–805 (2019).
9. Eerdekens, A. *et al.* Evolution of circulating thyroid hormone levels in preterm infants during the first week of life: Perinatal influences and impact on neurodevelopment. *J. Pediatr. Endocrinol. Metab.* **32**, 597–606 (2019).
10. Dereymaeker, A. *et al.* The suppression curve as a quantitative approach for measuring brain maturation in preterm infants. *Clin. Neurophysiol.* **127**, 2760–2765 (2016).
11. Niemarkt, H. J. *et al.* Quantitative analysis of maturational changes in EEG background activity in very preterm infants with a normal neurodevelopment at 1year of age. *Early Hum. Dev.* **86**,

219–224 (2010).

12. Niemarkt, H. J. *et al.* Maturational Changes in Automated EEG Spectral Power Analysis in Preterm Infants. *Pediatr. Res.* **70**, 529–534 (2011).

13. Jennekens, W. *et al.* Topography of maturational changes in EEG burst spectral power of the preterm infant with a normal follow-up at 2 years of age. *Clin. Neurophysiol.* **123**, 2130–2138 (2012).

14. O'Toole, J. M., Boylan, G. B., Vanhatalo, S. & Stevenson, N. J. Estimating functional brain maturity in very and extremely preterm neonates using automated analysis of the electroencephalogram. *Clin. Neurophysiol.* **127**, 2910–2918 (2016).

15. Meijer, E. J. *et al.* Functional connectivity in preterm infants derived from EEG coherence analysis. *Eur. J. Paediatr. Neurol.* **18**, 780–789 (2014).

16. Lavanga, M. *et al.* A brain-age model for preterm infants based on functional connectivity. *Physiol. Meas.* **39**, 44006 (2018).

17. De Wel, O. *et al.* Complexity Analysis of Neonatal EEG Using Multiscale Entropy : Applications in Brain Maturation and Sleep Stage Classification. *Entropy* **19**, 516 (2017).

18. Khedr, E. M., Toony, L. F. El, Tarkhan, M. N. & Abdella, G. Peripheral and Central Nervous System Alterations in Hypothyroidism: Electrophysiological Findings. *Neuropsychobiology* **41**, 88–94 (2000).

19. Santana-Miranda, R. *et al.* Cyclic alternating pattern in infants with congenital hypothyroidism. *Brain Dev.* **41**, 66–71 (2019).

20. Atkinson, R. *et al.* Biochemical, behavioural and electrophysiological investigations of brain maturation in chickens. *Brain Res. Bull.* **76**, 217–223 (2008).

21. Dereymaeker, A. *et al.* An Automated Quiet Sleep Detection Approach in Preterm Infants as a Gateway to Assess Brain Maturation. *Int. J. Neural Syst.* **27**, 1750023 (2017).

22. Costa, M., Goldberger, A. L. & Peng, C.-K. Multiscale entropy analysis of biological signals. *Phys. Rev. E* **71**, 1–18 (2005).

23. Humeau-Heurtier, A. The Multiscale Entropy Algorithm and Its Variants: A Review. *Entropy* **17**, 3110–3123 (2015).

24. Lu, W. Y. *et al.* Multiscale entropy of electroencephalogram as a potential predictor for the prognosis of neonatal seizures. *PLoS One* **10**, e0144732 (2015).

25. Kang, H. G. & Dingwell, J. B. Differential Changes with Age in Multiscale Entropy of Electromyography Signals from Leg Muscles during Treadmill Walking. *PLoS One* **11**, e0162034 (2016).

26. Busa, M. A. & van Emmerik, R. E. A. Multiscale entropy: A tool for understanding the complexity of postural control. *J. Sport Heal. Sci.* **5**, 44–51 (2016).

27. Benjamini, Y. Simultaneous and selective inference: Current successes and future challenges. *Biom. J.* **52**, 708–21 (2010).

28. De Vries, L. S., Heckmatt, J. Z., Burrin, J. M., Dubowitz, L. M. & Dubowitz, V. Low serum thyroxine concentrations and neural maturation in preterm infants. *Arch. Dis. Child.* **61**, 862–866 (1986).

29. Ng, S. M. *et al.* An explanatory randomised placebo controlled trial of levothyroxine supplementation for babies born. *Trials* **14**, 211 (2013).

30. Ng, S. M. *et al.* Effect of thyroxine on brain microstructure in extremely premature babies: magnetic resonance imaging findings in the TIPIT study. *Pediatr. Radiol.* **44**, 987–996 (2014).

31. Hüning, B. *et al.* Relationship between brain function (aEEG) and brain structure (MRI) and their predictive value for neurodevelopmental outcome of preterm infants. *Eur. J. Pediatr.* **177**, 1181–1189 (2018).

32. Williams, F. L. R. *et al.* Developmental Trends in Cord and Postpartum Serum Thyroid Hormones in Preterm Infants. *J. Clin. Endocrinol. Metab.* **89**, 5314–5320 (2004).

33. Jaume, J. C., Mendel, C. M., Frost, P. H., Greenspan, F. S. & Laughton, C. W. Extremely Low Doses of Heparin Release Lipase Activity into the Plasma and Can Thereby Cause Artifactual Elevations in the Serum-Free Thyroxine Concentration as Measured by Equilibrium Dialysis.

Thyroid **6**, 79–83 (1996).

34. van Wassenaer, A. G. *et al.* Effects of Thyroxine Supplementation on Neurologic Development in Infants Born at Less Than 30 Weeks' Gestation. *N. Engl. J. Med.* **336**, 21–26 (1997).

35. Willhelm, C. *et al.* Systematic Cochrane Reviews in Neonatology: A Critical Appraisal. *Pediatr. Neonatol.* **54**, 261–266 (2013).

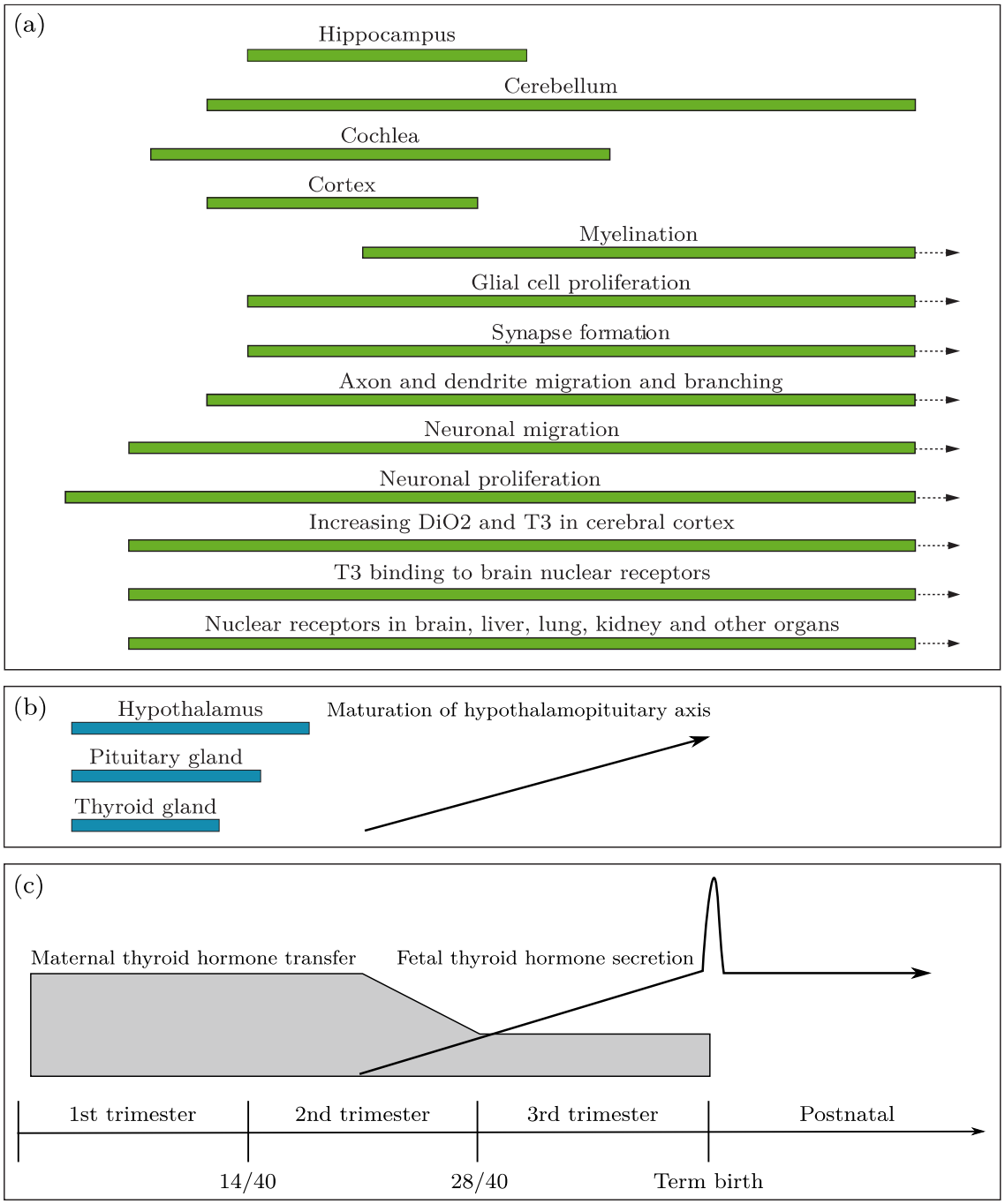

Figure 1: Fetal brain development in relation to maternal thyroid hormone supply and fetal thyroid hormone metabolism. (a) Embryological and fetal development of brain structures; (b) Embryological and fetal formation and maturation of thyroid hormone system structures; (c) Until mid-gestation, the fetus is completely dependent on maternal thyroid hormone supply. Thereafter, the fetal thyroid system starts to work, but it is only after birth that the infants' thyroid system functions completely autonomously. DIO2: type 2 deiodinase, T3: triiodothyronine. Adapted from [4].

Table 1: Patient characteristics

	Positive ΔfT4 (n=27)	**Negative ΔfT4 (n=36)**	**p-value**
Median GA (w) (+/- IQR)	26 (1)	26 (2)	0.007
Mean birth weight (g) (+/- SD)	910 (213)	834 (170)	0.2
IUGR (n) (%)	4 (14)	3 (8)	0.4
Median CRIB score (range)	4 (1-15)	5 (1-12)	0.1
Treatment with levothyroxine (n) (%)	4 (14)	20 (55)	0.001
BPD (n) (%)	7 (25)	18 (50)	0.07
ROP (n) (%)	8 (28)	15 (41)	0.4
PDA (n) (%)	11 (40)	25 (69)	0.04
Sepsis (n) (%)	9 (32)	20 (55)	0.1
NEC (n) (%)	1(3)	5 (14)	0.2
Cerebral lesions (n) (%)	5 (18)	16 (43)	0.04
IVH & infarcts (n) (%)	4 (14)	9 (24)	0.4
Persistent flaring (n) (%)	1 (3)	7 (19)	0.1

GA: gestational age; w: weeks; IQR: interquartile range; g: gram; SD: standard deviation; n: number; IUGR: intrauterine growth restriction; CRIB score: Clinical Risk Index for Babies (birth weight ≤ 1500 g and or gestational age < 31 weeks), range 0-23; BPD: bronchopulmonary dysplasia, defined as O2 and/or ventilation need at 36 weeks postmenstrual age; ROP: retinopathy of prematurity with need for lasertherapy; PDA: persistent ductus arteriosus; sepsis: positive blood culture; NEC: necrotizing enterocolitis, defined as Bell's stage II & III; cerebral lesions: grade II-IV intraventricular haemorrhage (IVH), persistent flaring > 2 weeks and cerebral infarcts.

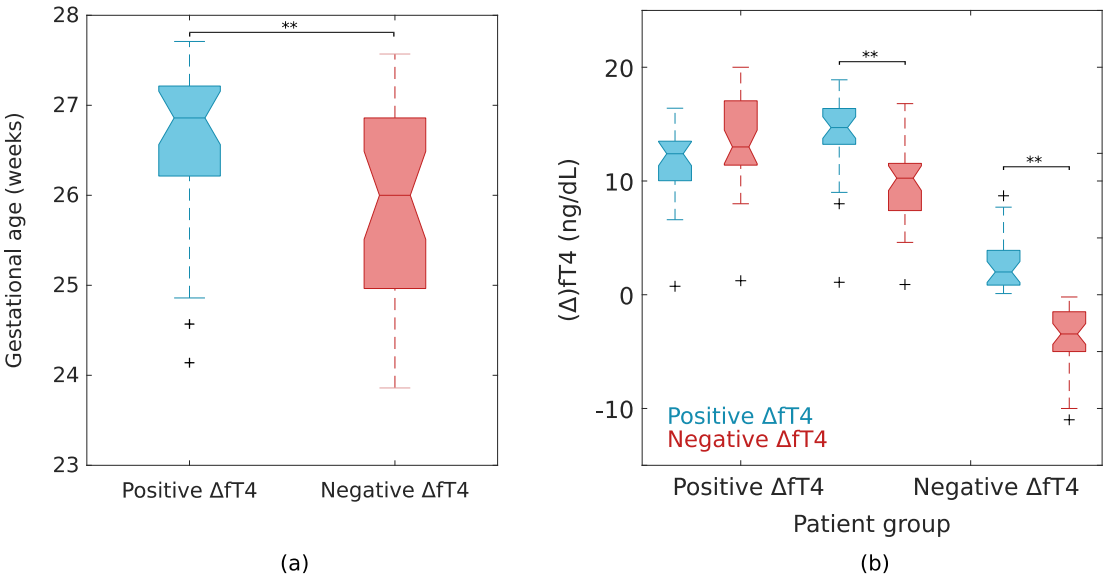

*Figure 2: (a) Boxplot of the gestational age of infants with positive ΔfT4 and negative ΔfT4. The gestational age is significantly lower in patients with negative ΔfT4 as indicated by '**' ($p < 0.01$). (b) Comparison of fT4 levels at day of birth, at the end of the first week of life and the difference between the two measurements for the patients with positive ΔfT4 (blue) versus patients with negative ΔfT4 (red). Both fT4 level at day 7 and the change in the first week is significantly lower in patients with negative ΔfT4 ($p < 0.01$, marked as '**').*

Table 2: Results of the mixed-effects model. For each maturational EEG feature, the regression coefficient indicating the association between the EEG feature and delta fT4 is set out ($b_{\Delta fT4}$). Moreover, the lower and upper bounds of the confidence interval of the regression coefficient (CI(lower;upper)) and its p-value are presented.

EEG feature	**b_{fT4}**	**CI(lower;upper)**	**p-value**
Complexity index	0.0423	(-0.0649;0.1495)	0.4383
Average slope small scales	0.0001	(-0.0009;0.0012)	0.796
Maximum MSE curve	0.0001	(-0.0002;0.0003)	0.5519
Relative power delta1	0.0029	(-0.0031;0.0088)	0.3477
Relative power delta2	0.0008	(-0.0018;0.0033)	0.5575
Relative power theta	0.0009	(-0.0018;0.0035)	0.5184
Relative power alpha	0.0002	(-0.0019;0.0022)	0.8828
Relative power beta	-0.0007	(-0.002;0.0005)	0.2471
SEF75	-0.0003	(-0.0007;0.0001)	0.1297
SEF90	0	(-0.0004;0.0005)	0.9755

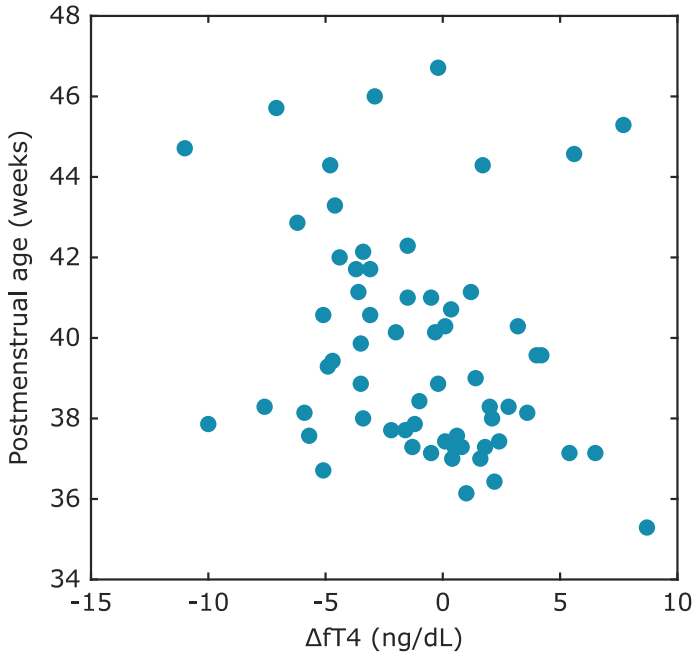

Figure 3: Postmenstrual age at which EEG time series were measured versus the change in free thyroxine concentration during the first week of life. It is clear that the electrocortical activity of patients with a decrease in fT4 level are measured at a later age, compared to neonates with a positive trend in fT4.