FULL-LENGTH ORIGINAL RESEARCH

Effects of prenatal antiepileptic drug exposure on newborn brain activity

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Summary

Objective: **Prenatal exposure to antiepileptic drugs (AEDs) is associated with an increased risk of cognitive dysfunction at early school age. Our aim was to investigate whether signs of adverse drug effects on brain function could be detected already during the first 2 weeks of life.**

Methods: **We studied prospectively 56 full-term newborns with prenatal exposure to AEDs and 67 unexposed newborns for the following characteristics: Background information, AED exposure data, pregnancy outcome, neuropsychological evaluation of the mothers, clinical neurologic status with Hammersmith Neonatal Neurological Examination and early cortical activity using electroencephalography (EEG). For EEG assessment, we developed and provide automated quantitation algorithms of several earlier described features: oscillatory bouts at theta and alpha frequencies, frequency spectra, interhemispheric synchrony, and interburst intervals (IBIs).**

Results: **The AED-exposed newborns had lower limb and axial tone and were less irritable than the unexposed newborns. EEG assessment disclosed significant differences in alpha bouts, in the frequency spectra, as well as in the spatial distributions of interhemispheric synchrony and IBIs.**

Significance: **The results indicate that fetal AED exposure may affect early neonatal neurologic status and several features of early cortical activity. The findings suggest that interference of activity-dependent network development may be a possible mechanism to explain the link from fetal AED exposure to later neurocognitive sequelae.**

KEY WORDS: **Epilepsy, Pregnancy, Neonatal, EEG.**

Epilepsy affects about 0.3–0.7% of pregnant women.[1] Most of them need antiepileptic drug (AED) treatment during pregnancy because seizures pose a risk to both the mother and the fetus.[2,3] Fetal AED exposure is known to increase the risk for major malformations and pregnancy complications, and the risk profile varies depending on AED and dose.[4–6] In addition to the major malformations detected prenatally or soon after birth, prior studies have suggested neurodevelopmental and behavioral consequences from fetal AED exposure.[5,7–9] These can be reliably detected earliest at 5–6 years of age, when numerous confounding factors have already exerted their effect and the causal relationship to fetal AED exposure is difficult to sort out.

The molecular and cellular mechanisms targeted with AEDs are diverse. Neurodevelopmental and behavioral effects of intrauterine AED exposure may relate to any of the complex processes of early brain development, including neuronal proliferation and migration, apoptosis, axonal and dendritic arborization, synaptogenesis, and finally myelination.[10] Experimental work has shown direct neurotoxicity by AEDs on the early network development.[11–13] It

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Key Points

- Fetal AED exposure may alter brain activity of the newborn
- Fetal AED exposure may interfere with temporal occurrence and spatial coordination of the developing brain and the tone of the newborn
- Neurophysiologic changes suggest mechanisms of adverse effects of fetal AED exposure
- Assessment during the early neonatal period may be less confounded by environmental factors than the results from longer follow-up

is notable, however, that the main actions of AED relate to modifying neuronal activity, the key factor in early neuronal survival and organization of neuronal networks.[12–15] It is hence conceivable that developmental adversities of AED may arise via early activity-dependent mechanisms.

Neuronal activity measured with noninvasive scalp electroencephalography (EEG) is a widely used method to assess early brain development.[16,17] Recent progress in understanding the relationship between neonatal EEG and its underlying network and molecular level mechanisms has improved the clinical and scientific information gain of the neonatal EEG.[18] The conventional visual review of full-term EEG includes assessment of the temporal continuity, spatial organization including synchrony, as well as observation of many visually salient EEG events, such as frontal sharp waves, anterior slow dysrhythmia, or bouts of alpha and theta frequency rhythms.[16,19,20] Changes in early EEG activities may correlate with later neurodevelopmental compromises, even without clinical signs of encephalopathy.[19,21,22] In addition, quantitative EEG methods are increasingly used in scientific work when estimating effects of early medical adversities, medications, or other therapeutic interventions on brain function.[21,23,24]

This study was designed to evaluate whether prenatal AED exposure could affect early brain development. In light of the above, it is conceivable that the neurodevelopmental consequences of fetal AED may be optimally traced by assessing early brain function. Therefore, we looked for possible signs of adverse drug effects in both neonatal neurologic function and early cortical activity after fetal AED exposure.

Methods

Data collection

The study was conducted in the Helsinki University Hospital (HUH). The ethics committee of the HUH has approved the study. The HUH adult epilepsy clinic serves as a tertiary epilepsy center for the HUH area population of 1.5 million inhabitants and is responsible for primary epilepsy diagnostics and treatment for all adult, 16- to 65-year-old Helsinki inhabitants, including all pregnant women with epilepsy (PWE) treated with AEDs living in Helsinki. Between October 2009 and December 2013, 118 PWE visited the HUH adult epilepsy clinic and were invited to participate in the present study. Of these, 12 (10%) had miscarriages or abortions. Of the remaining 106 pregnancies leading to live births, 43 were excluded from the study: 20 due to mother's refusal, 7 because of insufficient language skills to participate, 7 because of unavailability for follow-up due to living arrangement or planned move to another hospital district, 2 because the mother had nonepileptic seizures, 2 because of alcohol or drug abuse, and 3 due to unstable use of AEDs.

Of the 63 included pregnancies, 3 parents later withdrew consent. Four newborns were excluded because of prolonged hospital treatment due to malformation (n = 2), adrenal medullary neuroblastoma (n = 1), or prematurity (n = 1), resulting in 56 newborns completing the study. One mother was enrolled for two consecutive pregnancies, and there was one twin pregnancy. A cohort of 67 pregnant women with no AED or other brain-acting medication was recruited by a nurse during regular pregnancy monitoring in an outpatient clinic or by a newspaper announcement. Written informed consent was obtained from all study and control mothers.

Among the recruited pregnancies, 13 women (20.6%) had undergone surgery due to drug-resistant epilepsy (n = 9), arteriovenous malformations (n = 2), or abscess (n = 1). Of the pregnancies that were not recruited, two women (4.5%) had undergone surgery. Of the PWE completing the study, 20 (36%) had epilepsy of unknown, 23 (41%) with structural, and 13 (23%) with genetic (idiopathic) etiology. In the monotherapy group, the numbers of unknown, structural, and idiopathic etiologies were 17 (45%), 10 (25%), and 12 (30%), respectively. Newborns recruited in the study were exposed to AED polytherapy during pregnancy in 31.7% and newborns not participating in the study in 16.3% of the pregnancies (Table 1). All recruited pregnancies with valproic acid (VPA) had VPA monotherapy. The polytherapy regimens including VPA were received in pregnancies not recruited (n = 5), or not completing the study (n = 2) (Table 1).

No episodes of status epilepticus occurred in the study population. Convulsive seizures were reported in 3 pregnancies (5.4%), and other seizures (dyscognitive or limited to autonomic sensory or simple motor phenomena) in 18 pregnancies (32%). The frequency of PWE having nonconvulsive seizures was 46% in the polytherapy group and 11% in the monotherapy group during the whole pregnancy. Convulsive seizures during the first and second trimesters were limited to one among the PWE receiving monotherapy and two among the polytherapy group, but during the third trimester there were no convulsive seizures in the monother-

Table 1. AED exposure

	OXC/CBZ N (%)	VPA N (%)	LTG N (%)	LEV N (%)	TPM N (%)	Polytherapy N (%)	All N (%)
All recruited	20 (31.7)	5 (7.9)	10 (15.9)	7 (11.1)	1 (1.2)	20 (31.7)	63 (100)
/study completion	/19 (33.9)	/5 (9.0)	/8 (14.3)	/7 (12.5)	/1 (1.8)	/16 (28.6)[a]	/56 (100)
Not recruited	25 (58.1)	4 (9.3)	4 (9.3)	2 (4.7)	1 (2.3)	7 (16.3)	43 (100)
All	45 (42.5)	9 (8.5)	14 (13.2)	9 (0.5)	2 (1.9)	27 (25.5)	106 (100)

CBZ, carbamazepine; OXC, oxcarbazepine; LTG, lamotrigine; LEV, levetiracetam; VPA, valproic acid; TPM, topiramate; CZP, clonazepam; CLB, clobazam.
[a]Polytherapy combinations: LTG + LEV (n = 3), CBZ + LEV (n = 3), CBZ + TPM (n = 1), OXC + LTG (n = 1), OXC + LEV (n = 1), OXC + GBP (n = 1), OXC + CLB (n = 1), LTG + OXC + CZP (n = 1), LTG + LEV + CZP (n = 3), and LTG + TPM + CLB (n = 1).

apy group, whereas three PWE had convulsive seizures at that time. The number of seizures during each trimester did not differ significantly between different etiologic groups in the monotherapy group.

All children were born between April 2010 and May 2014. Background information, exposure data (including daily doses and serum levels for oxcarbazepine, carbamazepine, valproic acid, lamotrigine, and levetiracetam) and pregnancy outcome data were obtained prospectively at outpatient visits taking place every trimester and postnatally (Table 2 and Data S3).

Neurologic examination

The newborns were examined using the Hammersmith Neonatal Neurological Examination scale (HNNE)[25] by an experienced pediatric neurologist (MV) at 41–42 weeks of conceptional age (CA). We converted the raw scores of the 34 HNNE items to Optimality Scores taking into account the CA of the newborn (CA 39–40/CA 41–42). The six categories of Compound Optimality Scores (COS) and the Total Optimality Score (TOS) were calculated according to the Dubowitz.[25] The COS categories represent different aspects of newborn neurologic assessment (Tone, Tone patterns, Reflexes, Movements, Abnormal signs and Orientation, and Behavior). At the time of the EEG and neurological examination, 48 (86%) of the AED exposed newborns were at least partially breast-fed, 4 (7%) received no breast-feeding, and data was missing for 4 (7%) of the exposed newborns. The number of breast-fed newborns did not differ statistically between the exposed and unexposed newborns ($p = 0.42$).

Neurocognitive evaluation of the mothers

Neurocognitive evaluation[26–28] of the 48 exposed mothers and of 20 unexposed mothers was assessed by TN 7–12 months postpartum. In addition, two of the exposed mothers were evaluated by the same neuropsychologist within 7 years of the birth of the included infant as part of a clinical epilepsy assessment. There were no significant differences in verbal, performance, or clinically relevant executive functioning skills between the mothers in the AED and the control groups (Table 2).

EEG

The EEG signals were collected at sampling frequency $Fs = 250$ or 500 Hz using NicOne EEG amplifier (Cardinal Healthcare/Natus, U.S.A.) and EEG caps (sintered Ag/AgCl electrodes; Waveguard, ANT-Neuro, Germany) with 20–32

Table 2. Background information

	AED (n = 56)	Controls (n = 67)	Sig. (AED vs. controls)
GA,[a] weeks, mean (range, SD)	40.1 (37.4–42.3, ±1.24))	40.4 (38.4–42.1, ±1.05)	0.21
CA[b] during EEG, weeks, mean	42.2 (40.3–44.4, ±0.91)	42.3 (40.0–43.7, ±0.75)	0.72
CA[b] during neurologic examination, weeks, mean	42.2 (40.4–44.4, ±0.87)	41.6 (39.1–43.6, ±1.07)	0.01
Enrollment (GA[a] weeks, mean)	7.20 (3–24, ±3.20)	15.20 (5–22, ±7.19)	0.02
Educational level of the mother[c] (median)	2 (1–3, ±0.59)	1 (1–2, ±0.43)	<0.001
Age of the mother, years, mean, years	32.0 (24.0–41.0, ±4.34)	32.52 (21.0–41.0, ±4.15)	0.39
Smoking during the third trimester (%)	4%	0%	0.13
Neuropsychology of the mothers			
VIQ (mean)	111 (69–137, ±13)	114 (97–134, ±10)	0.53
PIQ (mean)	117 (62–138, ±12)	122 (100–138, ±11)	0.07
Executive problems (no/slight)	76%/24%	90%/10%	0.32
Gender (male %)	59%	65%	0.53
Parity (mean)	2.0 (1–6, ±1.10)	2.1 (1–5, ±1.10)	0.49
Apgar at 1 min (median)	9 (7–10, ±0.52)	9 (2–10, ±1.57)	0.04
Folic acid amount during the 1st trimester (mean, mg)	3.1 (0.6–4.0, ±1.71)	3.0 (0–8.0, ±2.30)	0.25
Birth weight (grams)	3,456 (2,370–4,590, ±525)	3,705 (2,808–4,800, ±450)	0.02
Hemoglobin of the newborn	167 (127–199, ±18)	171 (127–214, ±22)	0.61

VIQ, Verbal Intelligence Quotient; PIQ, Performance Intellligence Quotient. [a]GA, gestational age (weeks); [b]CA, conceptional age (weeks); [c]Educational level: (1) = tertiary, (2) = secondary, (3) = primary, (4) = illiterate.

electrodes positioned according to the International 10–20 standard. For sleep state assessment according to standard criteria with EEG and polygraphic channels,[16] we also included the following polygraphic channels: chin electromyography (EMG), electrocardiography (ECG), eye movements, and respiratory sensors. There were no filters applied to the EEG signals during their collection, apart from the in-built anti-aliasing (low-pass) filter and the high-pass filtering property of our standard AC-coupled amplifier. Notably, these filters don't affect signal properties within the range of frequencies studied in our present work.

The first visual EEG review was done using NicoletOne Reader software, and we selected good quality EEG epochs without notable technical or biologic artifacts from the time periods: active sleep preceding quiet sleep (AS1; mean 6.4 min, interquartile range [IQR] 6.3 min), active sleep following quiet sleep (AS2; mean 4.9 min, IQR 3.3 min), and quiet sleep (QS; mean 5.9 min, IQR 4.7 min) for further analyses. Visual rating was determined by consensus agreement (MV and SV) when needed. The epochs were converted to European Data Format (EDF) to be quantitatively analyzed using custom scripts in Matlab (Version R2012b, MathWorks, Natick, MA, U.S.A.).

EEG analysis

Quantitative EEG assessment consisted of four well-known features of neonatal EEG: (1) oscillatory activities (hereafter called "bouts") at theta (temporal) and alpha (frontal) frequencies during AS1 and AS2; (2) frequency spectra during both AS and QS; (3) interhemispheric synchrony during QS; and (4) interburst intervals (IBIs) during QS. The alpha and theta bouts were counted visually as well. The overall rationale in EEG signal processing for alpha/theta bouts and IBI quantitation are shown in Figure 1. The full Matlab scripts used in the present study as well as the full pathway with intermediate steps of our data analyses are provided in Supporting Information (Fig. S1, Data S1, Data S2, Data S4, and Data S5).

Oscillatory bouts

Theta frequency bouts were analyzed from the symmetric bipolar derivations T3-T5 and T4-T6 (temporal theta [TT], shown with green lines on Fig. 1B), whereas alpha frequency bouts were analyzed from the symmetric bipolar derivations Fp1-F3 and Fp2-F4 (frontal alpha [FA], shown in red on Fig. 1B). An example of all signal transformation stages is shown on Figure 1D.

First, raw EEG epochs were pre-filtered in 4–6 Hz (TT) or 10–13 Hz (FA) frequency bands using a combination of low-pass and high-pass Butterworth filters of the seventh order with respective cutoff frequencies. Filtering was done offline in forward and backward directions to achieve zero-phase signal shifts. Second, amplitude envelopes were computed with Hilbert transform from the filtered EEG.[23,29] Third, amplitude envelopes were smoothed with a Savitzky-Golay method,[30] where sliding time window (tw) was set to be $tw = 2$ s and polynomial order (k) was $k = 6$ as reasoned by our earlier work on neonatal EEG.[29] Detection of respective oscillatory bouts was performed from these smoothed EEG amplitude envelopes by applying threshold at three times median of the given epoch, hence adapted for each baby and each EEG channel separately. Minimum duration of bouts was set to 0.4 s for TT, and 0.2 s for FA, because exceedingly short bouts were logically and empirically reasoned to be not physiologic.

From these detections, we computed following metrics for each baby: average bout duration, cumulative proportion of bouts out of the whole recording epoch, and bout frequency (number/min).

Interburst interval (IBI)

IBI analysis was done from four bipolar derivations (F3-P3, F4-P4, C3-O1, and C4-O2; depicted in Fig. 1B). First, the EEG signal was filtered using a combination of high-pass and low-pass filters (see above) with cutoff frequencies at 3 and 15 Hz, respectively (Fig. 1E). Second, the nonlinear energy operator (NLEO) was applied to band filtered EEG to improve the discrimination of bursts from the other ongoing EEG. We used Teager operator as the NLEO function in this study.[31] Output of NLEO was smoothed with Savitzky-Golay approach using the same settings as described earlier, and burst events were detected using a threshold as three times median of the NLEO values in whole EEG epoch. IBI durations ranging from 2 to 10 s were considered physiologically relevant and included in the analysis. Finally, we combined centrooccipital (C3-O1 and C4-O2) and frontoparietal (F3-P3 and F4-P4) derivations into C/O and F/P groups, respectively, and median as well as average IBI durations were calculated for C/O and F/P of each baby.

Interhemispheric synchrony was computed using a recently published quantitative method, Activation Synchrony Index (ASI), which was shown to be robust and compare well with traditional visual assessment.[24] ASI analysis was done from two symmetric pairs of bipolar derivations (shown in blue on Fig. 1B): frontoparietal (F3-P3 vs. F4-P4) and centrooccipital (C3-O1 vs. C4-O2), both of which are indicated to perform well in the analysis of interhemispheric synchrony.[24,32] The average of these two ASI estimates was taken as an overall interhemispheric synchrony. Calculations of ASIs were performed using 2.5-min–long EEG epochs in each infant.

Spectral analysis

Frequency spectra of whole EEG epochs (Fig. 1B) were computed from bipolar derivations using short-time Fourier transform as an average of 2 s windows with 50% overlap and weighted with Hamming window. Further analysis considered frequency range 0.5–10 Hz with 0.5 Hz frequency binning using the Goertzel approach, and further data

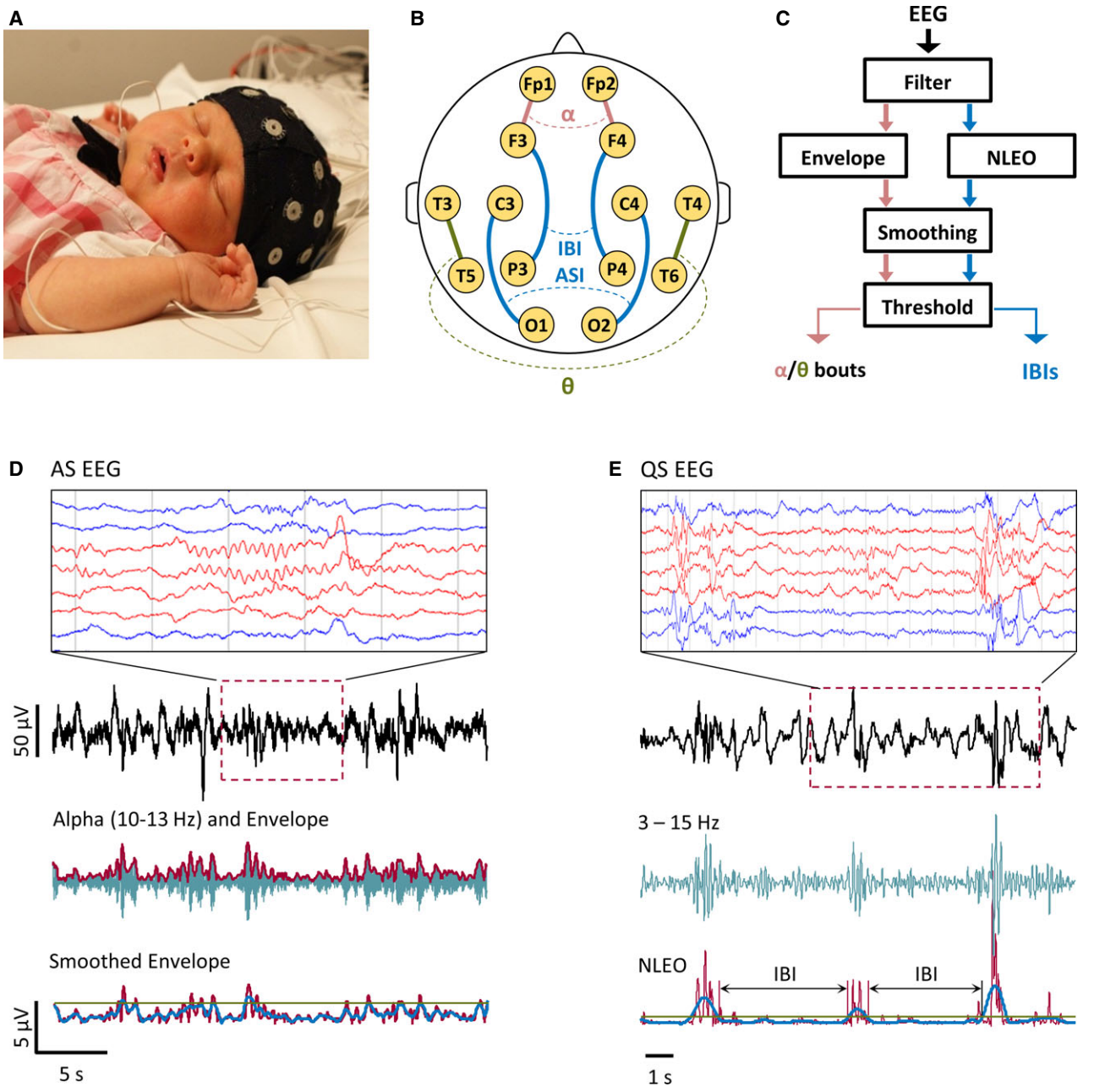

Figure 1.
Summary of EEG analysis. (**A**) Recording of EEG signal from a sleeping, full-term infant using EEG cap with 10–20 electrode layout. (**B**) Schematic drawing of the electrode placements used in our study. Colored bipolar derivations depict signals used for quantifying temporal theta (green), frontal alpha (red), and ASI or IBI (blue). (**C**) Schematic diagram of the analysis steps followed in our work. More detailed chart for data flow is shown in Supporting Information Figure S1. (**D**) Example of analytic transformations used for extracting theta and alpha bouts. (**E**) Example of analytic transformations used for quantifying IBI.
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reduction was done by averaging symmetric derivations (e.g. Fp1-F3 and Fp2-F4). In group comparisons, grand average spectra were computed from individually averaged spectra.

Statistical analysis

All investigations were carried out without the knowledge of the exposure status of the newborns. AED exposed and unexposed neonates were compared with each other. In addition, subgroup analyses were conducted between the neonates exposed to AED polytherapy or AED monotherapy and unexposed neonates. Comparisons were performed using nonparametric Wilcoxon signed-rank test (within individual) or Wilcoxon rank-sum test (between individuals). In cases with multiple comparisons, we applied differ-

ent corrections. When only a few comparisons were made (results from HHNE, oscillatory bouts, ASI), we applied Bonferroni correction. When a much higher number of statistical comparisons were performed (e.g., comparing spectral bins) correction for multiple comparisons was done as described[33]; 5% of significant observations from the total amount of tests of the weakest significant p-values was removed. The level of significance was set at $p < 0.05$.

Results

There were no significant group differences in the history of maternal cigarette smoking, alcohol consumption, folic acid supplementation, age, or parity. There were also no significant differences in the duration of pregnancy (gestational age, GA), hemoglobin level of the newborn, or in the conceptional age (CA) at the time EEG examinations were performed. However, during the neurologic examination there was a slight difference between the groups, as the mean CA of the AED-exposed newborns was 0.6 week higher than of the controls. Birth weight, 1-min Apgar scores, and educational level of the mother differed significantly between groups, although in our post hoc inspection, the difference in 1-min Apgar was found to be due to one outlier (Apgar = 2) in the group (Table 2). One newborn was referred to neonatal ward due to suspected neonatal jaundice. As per study design, recruitment was earlier in cases with AED exposure compared to control cases. There were no differences in neurologic or brain activity measurements between different etiologies of epilepsy or according to the occurrence of seizures.

Neurologic test (HNNE)

Several significant differences (summarized in Table 3) were found between AED and control groups in both the COS categories and TOS. Most importantly, the newborns exposed to AED had significantly higher COS for Tone and TOS. These results imply that exposed newborns had slightly lower limb and axial tone. There were differences between the AED group (as whole and monotherapy) and control groups in the score for Deviant Signs (including items of tremor, spontaneous startles, or abnormal hand posturing), but the difference was not seen between the polytherapy and control groups.

In the subgroup analyses, no differences in HNNE were found between newborns exposed to AED polytherapy versus monotherapy. However, a significant difference was seen between polytherapy and control groups in the COS for Tone (mean 5.14, standard deviation [SD] $\pm$ 2.25 vs. 7.33, SD $\pm$ 1.47, $p < 0.001$) and the TOS (22.7, SD $\pm$ 2.94 vs. 25.75, SD $\pm$ 0.35, $p = 0.011$).

Brain activity

Oscillatory events

Visual counting of bout numbers showed no differences between groups, and the automated quantitative analysis confirmed this with respect to TT. In contrast, detailed quantitation of FA bouts with automated analysis revealed several significant differences in EEG features not available in the visual analysis. First, the average duration of FA bouts ($p = 0.004$) and the percentage (the cumulative duration of FA to the epoch length) ($p < 0.001$) differed significantly between AS1 and AS2 in the Control group, whereas they did not differ significantly in the AED group (Fig. 2A, B). Comparison of different FA bout durations showed that newborns exposed to AED had fewer bouts with the typical medium duration than what was seen in the control group ($p = 0.03$) (Fig. 2C).

Interburst interval (IBI)

There were no differences in IBI lengths between AED and control groups. However, an uneven spatial distribution of IBIs was found only in the control newborns where IBIs were significantly longer in the posterior electrodes compared to frontal electrodes (median IBI 5.56 $\pm$ 0.79 s vs. 5.18 $\pm$ 0.53 s $p = 0.0071$; mean IBI 5.73 $\pm$ 0.59 s vs. 5.44 $\pm$ 0.39 s, $p = 0.01$).

Table 3. Effects of prenatal antiepileptic drug exposure on newborn neurology

	AED[a] (n = 56)	Mono[b] (n = 40)	Poly[c] (n = 16)	Controls (n = 67)	Sig. (AED[a] vs. controls)	Sig. (Mono[b] vs. controls)	Sig. (Poly[c] vs. controls)
Tone COS,[d] mean (SD)	6.03 (1.83)	6.35 (1.57)	5.14 (2.25)	7.33 (1.47)	<0.001	0.002	0.001
Tone patterns COS[d]	3.50 (0.86)	3.45 (0.90)	3.64 (0.74)	3.59 (0.68)	0.822	0.630	0.685
Reflexes COS[d]	3.93 (0.93)	3.85 (0.96)	4.20 (0.82)	3.78 (0.79)	0.214	0.486	0.092
Movements COS[d]	2.69 (0.45)	2.70 (0.46)	2.68 (0.42)	2.59 (0.50)	0.260	0.250	0.625
Deviant Signs COS[d]	2.56 (0.61)	2.55 (0.59)	2.58 (0.67)	2.84 (0.67)	0.007	0.007	0.157
Orientation and Behavior COS[d]	5.54 (1.05)	5.54 (1.05)	5.54 (1.10)	5.85 (1.02)	0.155	0.194	0.371
Total Optimality Score	24.06 (2.64)	24.4. (2.49)	22.7 (2.94)	25.75 (0.35)	0.007	0.035	0.110
Apgar	8.96 (0.52)	8.97 (0.59)	8.49 (1.43)	8.38 (1.57)	0.035	0.051	0.250

[a]Newborns exposed to AED, [b]newborns exposed to AED monotherapy, [c]newborns exposed to AED polytherapy. [d]COS = Compound Optimality Score of Hammersmith Neonatal Neurological Examination (HNNE) and Total Optimality Score (HNNE) according to Dubowitz 1999.

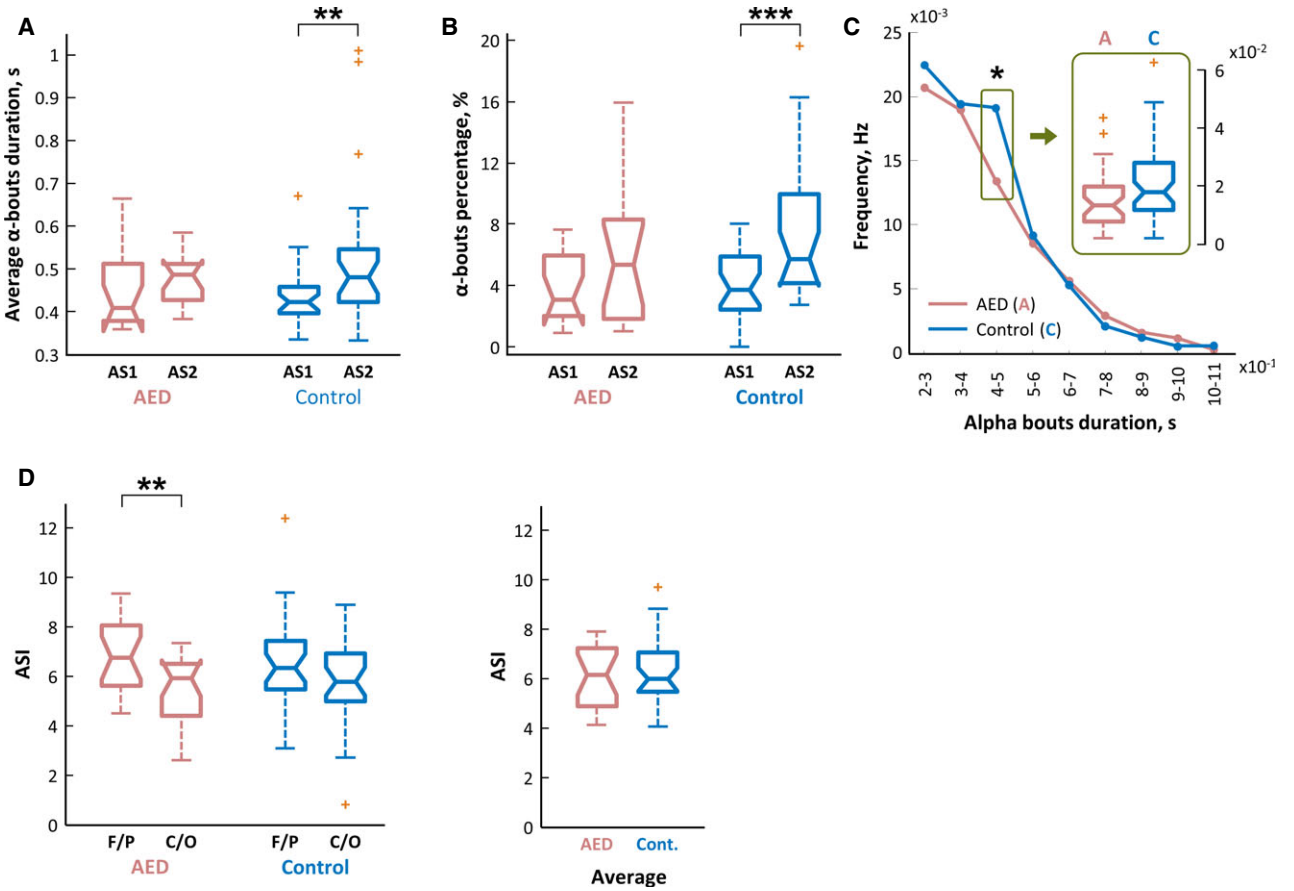

Figure 2.
Findings of oscillatory bouts and ASI analysis. There was a significant difference in average alpha bout durations (**A**) and percentage between the first and second active sleep epochs (**B**), but no difference between control and AED-exposed infants. (**C**) However, comparison of different bout durations showed that control infants had significantly more alpha bouts with duration of 400–500 msec. (**D**) Comparison of all ASI analyses showed no difference between control and AED-exposed infants. There was, however, a statistically significant intra-individual difference in the AED-exposed babies. F/P and C/O refer to bipolar derivations analyzed, frontoparietal and centrooccipital, respectively. Black asterisks show significant differences; red crosses show outliers.
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Interhemispheric synchrony

We found no significant difference in the ASI values between AED and Control groups in the frontal, posterior brain areas, or their average. However, infants exposed to AED showed a significantly higher ($p = 0.0025$) ASI in their frontal (6.81 ± 1.48) compared to posterior (5.49 ± 1.41) brain areas. No significant spatial difference was found in the control group (frontal 6.57 ± 1.83, posterior 5.81 ± 1.73, $p = 0.1766$) (Fig. 2D).

Spectral analysis

Comparisons of frequency spectra during both active and quiet sleep disclosed significantly lower amplitudes in the AED exposed infants at multiple derivations and frequencies (Fig. 3). The significant differences were mostly at lower delta frequencies, but they extended across most frequencies (1–10 Hz), especially during active sleep in the frontoparietal derivation. Subgroup analysis showed multiple significant differences in the lower frequency range when controls were compared with groups receiving monotherapy or polytherapy. Comparison of polytherapy and monotherapy groups to each other did not show statistically significant difference in any individual frequency bins; however, newborns exposed to monotherapy showed generally higher amplitudes throughout the frequency range.

Discussion

The present study shows that fetal AED exposure is associated with several neurologic and neurophysiologic changes that can be observed during the early neonatal period. Previous studies[5–9] have assessed AED effect on pregnancy outcome or developmental or behavioral influences later in life. To our knowledge, this is the first study that aims to disclose mechanisms underlying these effects. The

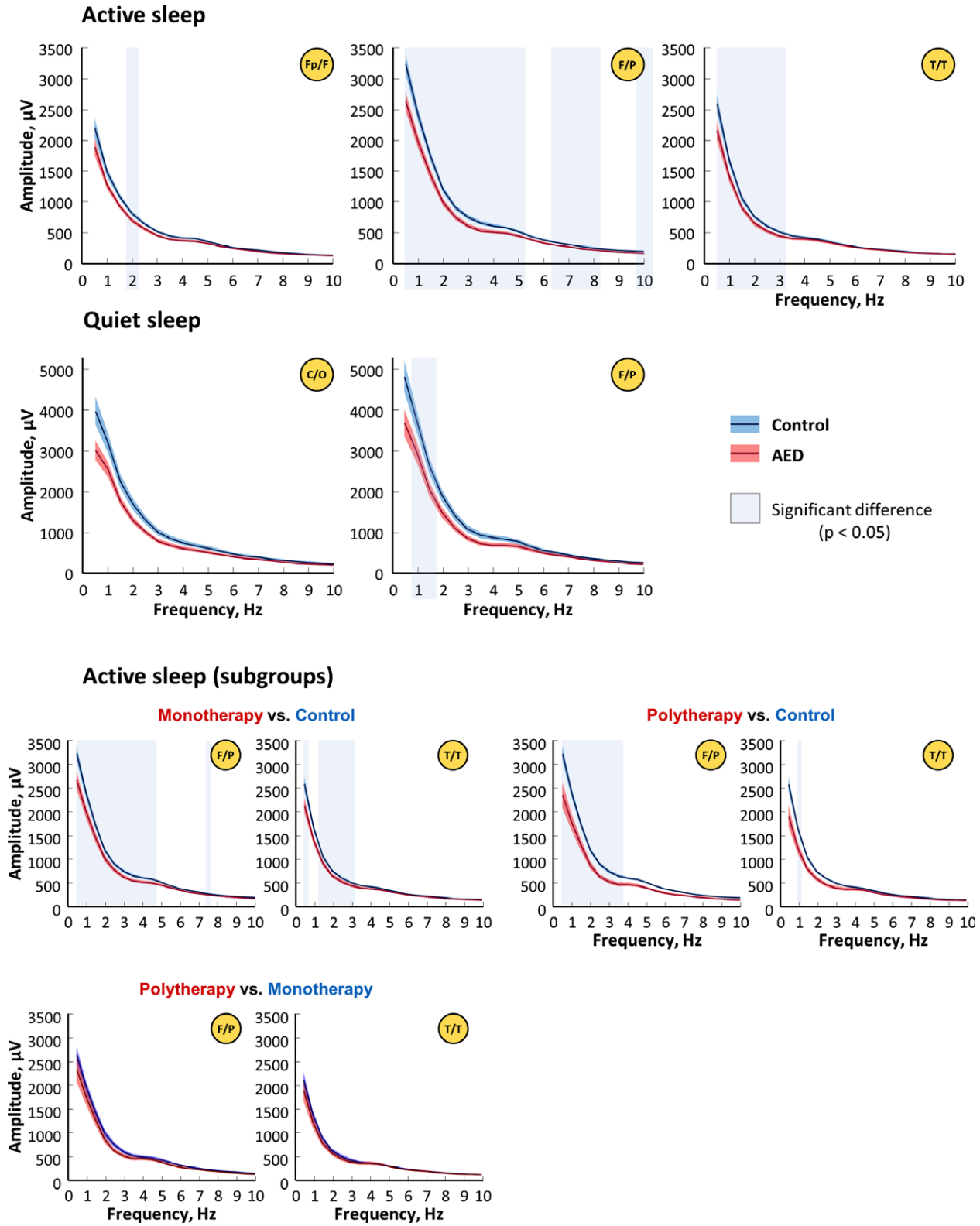

Figure 3.

Spectral analyses. Comparisons between AED and control groups in active and quiet sleep are shown on top for different bipolar derivations. Lower rows show comparisons between drug treatments. Gray shadows depict frequency bins with significant difference after correction for multiple comparisons. The derivations used are shown in the circle in the upper right corner of each spectrum plot.

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very early neurophysiologic changes investigated here are not susceptible to environmental confounders, which inevitably affect the results from longer developmental follow-up.

Clinical neurology

In line with earlier studies[34] showing increased apathy and hyperexcitability of the newborn after fetal exposure to phenobarbital, phenytoin, valproic acid, and benzodiazepines, our study implies that newer AEDs have similar effects expressed as lower axial and limb tone. This change in muscle tone could perhaps partly explain why AED-exposed newborns are more often admitted to the neonatal unit or require ventilatory support after birth than unexposed neonates.[1,6] Akin to earlier studies,[1,6] birth weight was lower in the AED group, although the amount of folate acid supplementation and the hemoglobin levels of the newborns did not differ between the control and the AED groups. Overall, the nutritional status of the pregnant women in Finland is considered good.[35]

Brain activity

Very few studies have been published on the EEG effects of acute drug exposure in the fetus or neonate.[36,37] Indeed, we are not aware of any prior studies on the effects of fetal exposure to the neonatal EEG activity. Our present work can be compared, however, to prior knowledge from other acquired or environmental challenges to brain development. Multiple studies have shown that a variety of clinically encountered conditions, such as early brain lesions, respiratory challenges, or metabolic diseases may affect brain activity in a visually obvious manner.[16,19,20,38] Moreover, environmental enrichment of the preterm baby may lead to measurable changes in the EEG.[39] Our present work extends these observations by showing that both individual oscillatory bouts and wider band spectra may be altered by fetal AED exposure. The developmental significance of these findings remains unknown, prompting further studies on the as-yet elusive developmental significance of individual oscillatory events. To facilitate further research in this direction, we provide here the full analysis code for use in other studies. Identification of such early EEG metrics does also hold promise for developing functional biomarkers for future studies on the developmental effects of drugs, neurologic adversities, or their treatments.

With regard to functional brain networking and its temporal organization, our findings suggest distinct changes by AED. We showed that newborns exposed to AEDs had higher frontal than posterior ASI, whereas control babies had longer IBI in posterior than frontal areas. Interhemispheric synchrony estimated with ASI value is by definition a measure of event coincidence, or spatial coordination, between hemispheres, whereas IBI is a measure of event occurrence. Hence, these two measures detect different aspects of the ways that the brain generates and coordinates early network events,[23] which are shown to be fundamentally important for early brain development.[18] Our present results cannot directly indicate their developmental significance; however, the results suggest that AED exposure may interfere with temporal occurrence and spatial coordination of brain mechanisms known to eventually guide brain wiring.

Strengths and limitations

All exposure data were gathered prospectively; neuropsychological evaluation of the mothers was conducted and outcome variables were measured using structured or quantitative methods at neonatal age before confounding factors take real effect. Our tertiary center cohort with exposure information also on all excluded pregnancies allows a better estimation of the recruitment bias than achieved in registry studies. Treatment-resistant epilepsy was likely to be overrepresented in the PWE recruited, since polytherapy was received twice as often as reported for all Finnish PWE during the study period.[6] The relatively small fraction of valproic acid exposures in both the recruited and not recruited groups is likely due to both avoidance of valproic acid because of the increasing information on teratogenic risks and overrepresentation of treatment-resistant focal epilepsy compared with more benign generalized epilepsy syndromes. Awareness of teratogenic risks may have decreased the willingness to participate of those pregnant women who were treated with polytherapy, especially with valproate (Table 1).

Despite of the prospective nature of recruitment, many questions related to causal relations of molecular mechanisms are difficult or impossible to answer with our present work or with any studies on human subjects. Although the overall effects of AEDs on newborn brain activity were clear, subgroup comparisons between drugs were limited by the number of newborns available in each group. It is not possible in the human studies to study whether maternal epilepsy per se could have some unidentified, direct effects on fetal development as opposed to the medicine used for treating the epilepsy.

The elimination half times of the AEDs included were <50 h, and as the EEG studies were measured in average at the age of 16 days, the drug levels in the newborn serums through transplacental transmission were considered to be extremely low. However, the metabolism of AEDs in the neonatal period is still not fully understood,[40] and most newborns in both groups were breast-fed, which may allow some postnatal AED exposure. Hence, we cannot exclude the possibility that some AED is still present in the infants during EEG recordings, which might partially account for the reported EEG effects. Unfortunately, the number of AED-exposed newborns without breast-feeding was too small to allow statistical comparisons.

Biologic considerations

The early brain activity, here measured using EEG, is known to be crucial for early brain wiring,[15,18] the foundation of all later neurocognitive functions. Our study suggests indirectly that one of the possible mechanisms for AED change of the cognitive or the behavioral development of the child could be this altered electrical activity of the neonatal brain. More studies are needed to evaluate if, for example, exposure to other medications, perinatal ischemia, or prematurity would display similar changes in brain activity.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The overall chart of the signal-processing pipelines that were used in this report.

Data S1. Matlab script for alpha/theta bouts detection.

Data S2. Matlab script for interburst intervals (IBI) detection.

Data S3. AED dosages and serum concentrations.

Data S4. Word transcript of Matlab code for alpha/theta bouts detection.

Data S5. Word transcript of Matlab code for interburst interval (IBI) detection.