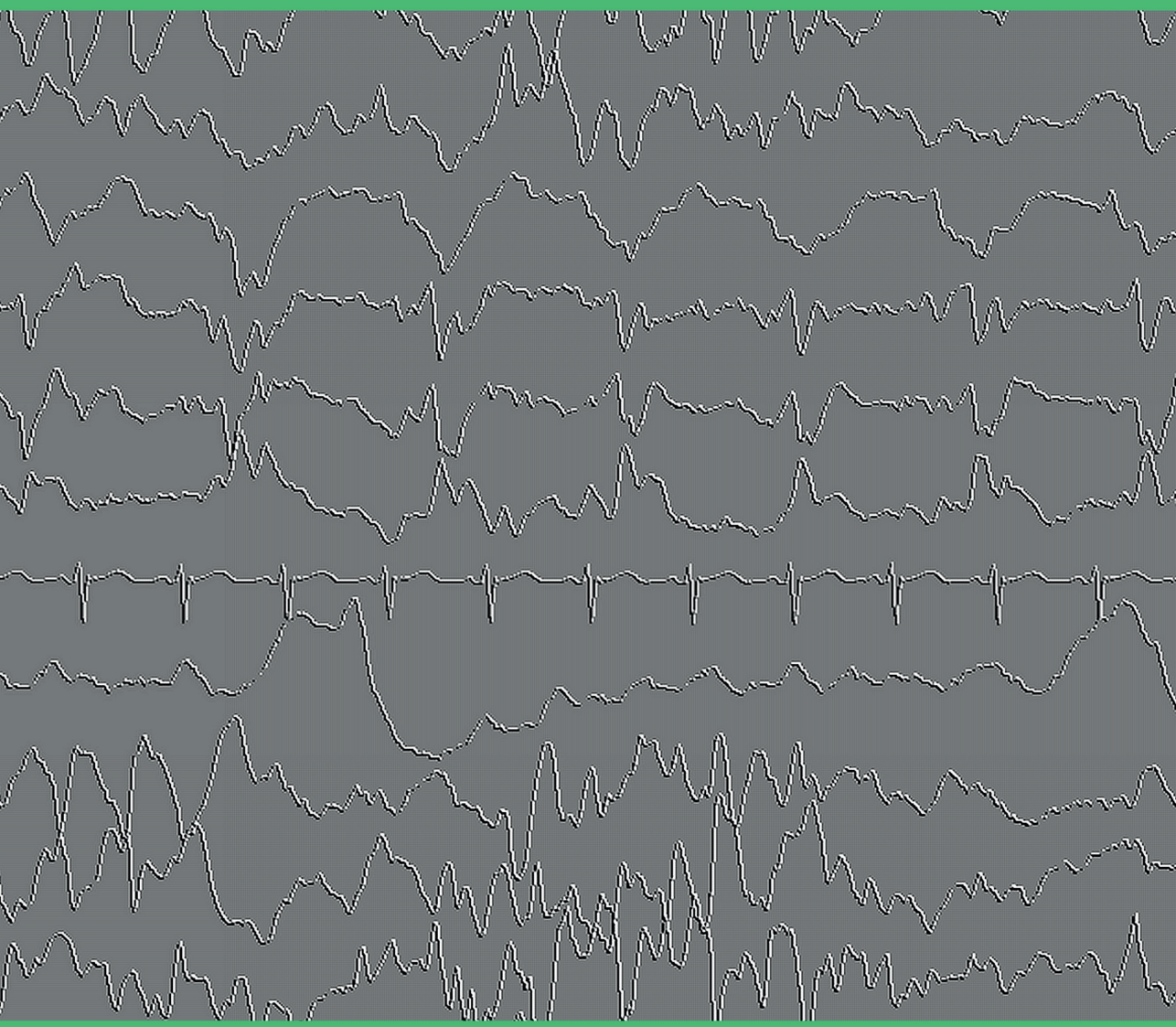
Improvements in Neonatal Brain Monitoring after Perinatal Asphyxia



Joseph Cherian Perumpillichira

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Improvements in Neonatal Brain Monitoring after Perinatal Asphyxia

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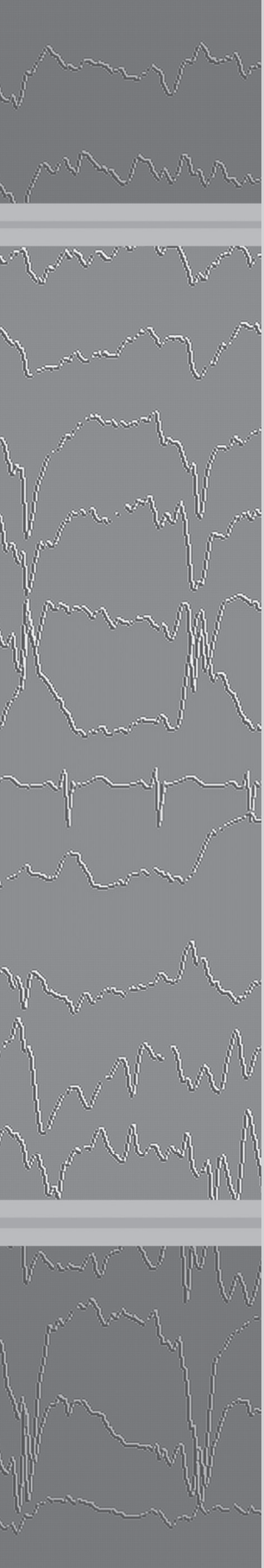

Chapter 1

Introduction

1.1 Pathophysiology of perinatal hypoxic brain injury and neonatal seizures, and their study using EEG and imaging

i) ***Perinatal brain injury due to hypoxia-ischemia***

Perinatal hypoxic ischemic encephalopathy (HIE) is a major cause of morbidity and mortality world-wide[1-3]. Common sequelae in survivors include cerebral palsy (CP), epilepsy and sensory as well as cognitive problems. The consequences of HIE impose significant long-term personal and financial burden on the affected families and the society. The most cost-effective approach to reducing neonatal mortality world-wide would be to improve access to antenatal care[4]. However, even in developed countries, the exact factors triggering perinatal asphyxia as well as the time of onset of brain injury are often difficult to determine, and it remains a major clinical problem[1,5]. Seizures commonly occur in the neonate with HIE and are often the only sign of serious underlying brain dysfunction[6]. Animal studies have shown that neonatal seizures in the context of HIE may cause additional brain injury[7,8] and that their pharmacological suppression may improve outcome[9]. Monitoring of brain function using the electroencephalogram (EEG), continuously[10] or by serial EEGs[11] is well-suited to give insight into brain function and its dynamic changes in neonatal HIE and helps to guide treatment as well as prognostication[12]. A good understanding of the pathophysiology of HIE is needed not only in the selection of suitable diagnostic tests and treatment methods, but also to develop new therapeutic strategies.

ii) ***Pathophysiology of hypoxic brain injury and its implications for clinicians***

The degree of hypoxic brain injury is mainly determined by the severity and duration of the insult[13] as well as the gestational age (GA) of the infant. Acute reduction in oxygen delivery to the brain results in break down of the neuronal energy metabolism within minutes. The susceptibility of newborn brain to hypoxia is also dependent on other factors like the presence of antioxidants and the number and distribution of excitatory N-methyl-D-aspartate (NMDA) receptors etc. The hypoxia-induced modification of the NMDA receptors (a type of ionotropic glutamate receptors) leads to increased intracellular calcium (Ca^{2+})[14], which activates proteases, phospholipases and nitric oxide synthase. Activation of these enzymes results in the generation of free radicals, peroxidation of nuclear membrane lipids and activation of endonucleases leading to fragmentation of nuclear DNA. These secondary changes are

responsible for failure of cerebral energy metabolism after a phase of apparent recovery and are associated with the appearance of epileptiform abnormalities on EEG.

Electrographic seizure discharges: A study in sheep fetuses showed that epileptiform abnormalities appeared about eight hours after 30 minutes of global ischemia and reached a peak after 10 hours[15]. Understanding the time course of these changes helps to optimize the timing and duration of brain monitoring using EEG.

Apoptosis: Hypoxia also induces via Ca^{2+} -dependent pathways, transcription of pro-apoptotic proteins. These proteins initiate programmed cell death by activating a family of proteases known as caspases, which in turn cleave intracellular structural proteins like actin and also inactivate nuclear enzymes. This sequence results in cell nucleus disruption, a characteristic feature of apoptosis.

Selective vulnerability of various brain regions: This is dependent on various factors like the GA, timing and severity of the insult as well as the metabolic activity and blood supply of the concerned anatomical region. Injury to periventricular white matter (periventricular leukomalacia) typically occurs in preterm babies, upto 34 weeks of GA and results predominantly in spastic diplegia type of CP[16]. However, extrapyramidal or dyskinetic CP, related to basal ganglia injury, is described more commonly in term perinatal asphyxia[17]. Basal ganglia and thalamus (TBG) injury is typically associated with severe 'sentinel' events at birth like placental abruption, and results in severe neurological impairment[18,19]. Repeated prenatal asphyxia in near-term fetal sheep, induced experimentally by episodes of umbilical cord occlusion, aimed to mimic established labor, resulted in varying degrees of injury to deep grey matter or to parasagittal cortex and cerebellum, depending on the interval between the occlusions[20]. Enhanced energy metabolism as well as presence of excitatory synaptic connections in the basal ganglia, hippocampus and perirolandic cortex are believed to make them more vulnerable to injury[21]. Systemic hypotension is likely to cause more injury to the watershed regions of the brain[22].

Neuroprotection: In cerebral tissue that is capable of recovery after a hypoxic insult, secondary injury develops over a time period of many hours, giving a potential 'therapeutic time window' during which neuroprotective strategies are expected to work[23]. The best studied and most promising among the neuroprotective strategies is therapeutic hypothermia. Increasing or decreasing the temperature by even 1 to 2 degrees Celsius markedly affects (worsens, or protects against, respectively) hypoxic brain injury[24]. In animal experiments on cerebral ischemia, hypothermia is consistently associated with dose-related, long-lasting neuroprotection[25]. Both selective head cooling[26] as well as whole body hypothermia[27,28] safely

decreased the combined outcome of death or disability at 12-24 months in infants with HIE. Various compounds that have shown promise, albeit of a modest nature, as neuroprotective agents in perinatal brain injury, include Magnesium in prematures[29], the antiepileptic drug (AED) Topiramate[30] and N-acetyl cysteine[31]. The combination of pharmacological agents with hypothermia may show a synergistic effect[32] in neuroprotection.

iii) ***The occurrence of seizures in HIE***

Seizures result from abnormal, paroxysmal, excessive, synchronous electrical discharges from a group of neurons in the brain. Neonatal period is the most vulnerable period during the lifetime for expression of seizures[33]. Various causes have been proposed for post-asphyxial neonatal seizures, like energy failure disturbing the physiological membrane potentials, excess of excitatory neurotransmitters etc.[6]. Seizures can induce changes in the brain, like increased glucose and energy substrate utilization and increased lactate levels, and lead to harmful short term and long-term effects[7,8]. A detailed description of the pathophysiology of seizures is beyond the scope of this chapter. It is however, important to realize that majority of neonatal seizures are acute provoked seizures, and that they do not recur after the neonatal period, except in a small percentage of patients[33]. These phenomena are manifestations of underlying acute brain dysfunction and are thus very different from epileptic seizures (recurrent, unprovoked seizures) seen in older children and adults. There is no convincing evidence that post-asphyxial neonatal seizures independently cause brain injury in humans. Different published studies, addressing this problem, have various methodological limitations[12,34-36]. The main reason why this evidence has so far eluded researchers, is that the effects of HIE are often difficult to separate from that of the seizures. Similarly, there is no evidence that treatment of post-asphyxial neonatal seizures with AED improves clinical outcome.

iv) ***The rationale for continuous EEG monitoring (cEEG) in the sick neonate***

EEG is a widely available and well-accepted non-invasive investigation that can be done at the bedside to study brain function. It shows high temporal resolution and is highly sensitive to detect changes in the functional and metabolic status of the brain as well as the occurrence of epileptic seizures[37,38]. It is possible to use cEEG to detect deleterious changes in brain function at an early stage, when it is potentially reversible. One of the major problems hampering research about HIE related seizures is the frequent occurrence of electro-clinical dissociation (‘subclinical’ seizures without or with only subtle external motor

manifestations)[39]. This means that in a NICU population, cEEG would be required to study seizures and the effect of their treatment. However, the expertise required for starting EEG registrations and interpreting them is not readily available in the NICU. This has prompted many centers to adopt simple compressed EEG trends like amplitude integrated EEG (aEEG) for brain monitoring. This single channel monitoring is useful in detecting changes in EEG background activity and some of the expressed seizures. However, it is easily distorted by external artefacts and fails to give spatial and morphological information about seizures. More detailed descriptions of cEEG and aEEG are given in the next chapter.

v) ***Automated detection of neonatal seizures***

A realistic solution for the implementation of cEEG for detection and treatment of subclinical seizures in the NICU, would be to develop automated methods for seizure detection. Various researchers have developed systems based on wavelet analysis[40], autocorrelation to detect rhythmic oscillations[41], frequency analysis and spike detection[42], a combination of the above methods with neural network-based classification and contextual rules, modelling and complexity analysis[43], non-linear EEG analysis[44] etc. These systems, with the exception of the method by Gotman[42] have not been tested on data from multiple centers. None of the above mentioned groups have developed a widely accepted seizure detection system, ready for use at the bed-side. In addition to detection of occurrence of seizures, research is also needed into other characteristics of seizures like their location, firing frequency, rhythmicity and spread, which may have a relationship to the type of brain injury. These parameters also need to be incorporated into an automated seizure detection algorithm.

vi) ***Neuroimaging findings in neonatal HIE and seizures***

MRI is highly sensitive in detecting hypoxic brain injury. On conventional studies (T1 and T2 weighted spin-echo images), involvement of basal ganglia and thalamus[45] as well as posterior limb of internal capsule[46] suggest a poor prognosis and high risk for developing psychomotor retardation[47]. Abnormalities on diffusion weighted imaging (DWI) and apparent diffusion coefficients (ADC), presumably caused by restricted diffusion of water molecules in the injured tissue, are present in the early stages of brain injury (first week of life)[48-50] and may normalise after that (pseudo-normalisation). Also, damage to basal ganglia and thalamus may be missed if only diffusion weighted studies are done, as apoptotic damage to these structures may lead to an increase rather than decrease in the ADC values, after the first few days of the hypoxic insult[49]. A few studies have looked at imaging findings in neonatal seizures[51,52], and

have described presence of both ischemic and hemorrhagic lesions, but none have done detailed correlation of brain lesions with seizures detected by cEEG.

vii) ***Aim of this thesis***

Aim of this thesis was to improve brain monitoring after perinatal asphyxia. Studies were focused on better understanding the EEG characteristics of neonatal seizures in relation to brain injury, as well as the development of an automated seizure detection system for long-term continuous EEG monitoring.

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1.2 Technical standards for recording and interpretation of neonatal electroencephalogram in clinical practice

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Ann Indian Acad Neurol 2009;12:58-70

ABSTRACT

Neonatal electroencephalogram (EEG), though often perceived as being difficult to record and interpret, is relatively easy to study due to the immature nature of the brain, which expresses only a few well-defined set of patterns. The EEG interpreter needs to be aware of the maturational changes as well as the effect of pathological processes and medication on brain activity. It gives valuable information for the treatment and prognostication in encephalopathic neonates. In this group, serial EEGs or EEG monitoring often gives additional information regarding deterioration/improvement of the brain function or occurrence of seizures.

INTRODUCTION

Electroencephalogram (EEG) offers a window to the brain function and has a unique place in the care of sick newborn babies. Its value is increased when neurological examination is confounded by the use of sedatives and neuromuscular blocking agents (even though EEG itself may be influenced by sedatives). It is done in a neonate commonly for the following indications: a) assess the severity of brain dysfunction, b) detect (subclinical) seizures, c) assess cerebral maturation, and d) determine prognosis.

To derive maximum benefit from an EEG, the neurologist needs to be familiar with maturational changes as well as the effect of various pathological conditions on the EEG. Often, the recording has to be made in the NICU, with interference from monitoring equipment, indwelling lines, incubator, and frequent nursing care. In this article, we highlight those technical details that are relevant for neonatal EEG registration and describe normal and abnormal EEG phenomena in the preterm and the term neonate.

Recording the EEG: Preparation, precautions, and technical aspects

Patient information

Age of the neonate: Knowledge of the exact age of the baby undergoing EEG is important for a proper interpretation. Maturational changes occur fairly rapidly in the 25–48 week period [Table 1] and any discrepancy in the age-related EEG findings of more than two weeks is abnormal. The gestational age (GA) is defined as the time elapsed between the first day of the last menstrual period of the mother and the birth of the baby.[1] A baby born prematurely at 32 weeks and having a chronological age of four weeks at the time of EEG recording is considered to have a postmenstrual age (PMA) of 36 weeks. This corresponds to a conceptional age (CA) of 34 weeks, assuming that conception occurred two weeks after the first day of the last menstrual period. However, in practice, the term CA is used interchangeably with PMA by many authors.

Clinical information: Time of birth, history of birth asphyxia, Apgar scores, occurrence of convulsions, etc. need to be noted by the technician before starting the recording.

Medication use: Medications like morphine, barbiturates, and benzodiazepines may influence the EEG findings,[2] especially by lowering the voltage of the background activity. Their dosages, time of administration, and serum levels, if known, should be noted.

State of the patient: Noting the condition of the neonate, whether awake or asleep, on a ventilator, lying in an incubator, etc. is relevant. This helps not only in relating EEG

phenomena to the state of the patient, but also in recognizing artefacts like those arising from high-frequency ventilation. Changes happening in the environment like loud noises, flashes of bright light, nursing care, etc. should also be noted, as these may produce transient attenuation of the background activity or produce movement artefacts.

Recording the EEG

The timing of registration after birth: If the EEG is done to assess the degree of brain maturation, it can be done at least 24 hours after birth to ensure that transient EEG abnormalities caused by birth itself are not recorded.[3] If however, the goal is to assess the degree of encephalopathy and detect subclinical seizures, in situations like perinatal asphyxia, it is advisable to start the EEG registration (as a part of serial EEGs or EEG monitoring) as early as possible (at least within 24 hours), after respiratory and hemodynamic stabilization.

The duration of recording: Recording with the child asleep as well as awake is needed for the proper interpretation of neonatal EEG. A record made after feeding the baby is likely to succeed in this. The preparation for recording, like pasting of electrodes, can be done toward the end of the wake period so as to ensure that the neonate is not disturbed during sleep.[3] The registration time should be 45 minutes or longer. Abnormal or absent sleep–wake cycling[4-6] is sometimes the earliest indicator of brain dysfunction, and in such situations, it is required to record the EEG for a longer period (at least two to three hours).

Adequate skin preparation is required to achieve a scalp impedance of <5 kΩ. Mildly abrasive pastes (like NuPrep ™ gel) applied using a cotton bud, followed by alcohol swabs generally give a good result. Enough time (at least 90 minutes) has to be scheduled for the EEG recording to avoid stressing the technician and the parents.[3] This is preferable to registering an artefact-filled EEG, and later trying to minimize their influence, for instance by changing the filter settings.

Electrodes and montages

At our center, we use the full 10–20 system of electrodes for all neonatal EEGs done during working hours and the restricted system of electrode placement during emergency EEGs done outside the working hours. The restricted 10–20 system of electrode placement uses nine active scalp electrodes – Fp_{1-2}, Cz, C_{3-4}, T_{3-4}, and O_{1-2} electrodes.[3] Important spatial information is lost by using lesser number of electrodes. However, most clinical indications for an emergent EEG at this age do not call for a high degree of spatial resolution. Assessment of background activity is not affected by the reduced number of electrodes. The reduced montage has been shown to have a high sensitivity (96.8%) and 100% specificity when

compared to a full 10–20 montage in detection of neonatal seizures.[7] Silver–silver chloride EEG electrodes with conductive adhesive electrode paste (like 10–20™ paste) are used. In addition, at our center, we prefer using 3% collodion with small pieces of cotton or gauze for optimal fixation of the electrodes. This gives a better quality registration and also ensures that there is no deterioration of electrode contact if the recording has to be extended to continuous EEG monitoring. In a period of more than 25 years of using this method, we have not encountered any allergic skin reactions or hazards due to flammability. Compressed cold air is used for drying. Fire hazard due to the flammable collodion has to be borne in mind, and a hair dryer should never be used. Removal of the electrodes is done using an acetone-free solvent (we use collodion-remover from Mavidon™ Medical Products). Acetone works equally well, but its fumes are quite irritating. We use the same technique in a baby lying in an incubator. However, the incubator is kept open for sometime after fixing the electrodes.

Many EEG transients have a preponderance at the vertex, and hence, inclusion of the Cz electrode as well as a coronal bipolar electrode derivation in addition to the bipolar anteroposterior derivation is meaningful [Figure 1]. With digital EEG equipment, montage selection during registration of EEG is no longer very important.

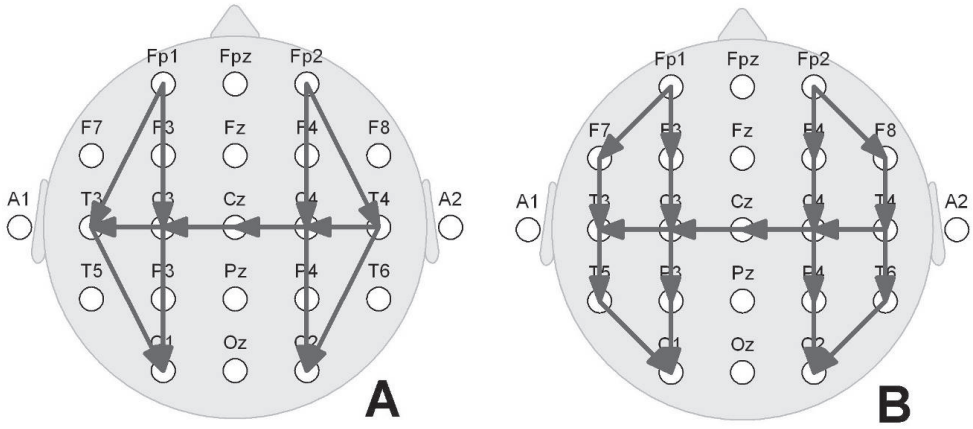


Fig 1. Neonatal EEG montages. **A**: Restricted 10–20 system using nine electrodes; **B**: Full 10–20 system of electrode placement using 17 electrodes. Note the bipolar derivations: anteroposterior right and left, as well as a coronal run including the central midline

Electrode caps: They help to save time, especially when a full 10–20 system of electrodes, or a more extensive recording (e.g., high-density EEG) has to be made. They also help to keep the electrode positions fairly accurate, especially in centers where there are no experienced technicians. The quality of the record depends on a snug fit, and thus, a wide range of cap sizes fitting different head sizes would have to be available. The electrode positions may show some change over time and with repeated use, much more than with conventional electrode application.[8]

Filters: Very slow waves (0.2–0.5 Hz) occur in the neonate and hence the low-frequency filter is set at 0.005–0.01 Hz. This very low filter setting may make the EEG more vulnerable to slow artefacts like sweat potentials. The high-frequency filter is set at 70 Hz.

Polygraphy: Polygraphic registrations are the rule while recording neonatal EEG[3]. Simultaneous recording of video is also becoming a standard practice. Video helps in assessing clinical seizures and in recognizing artefacts. Physiological variables like eye movements, ECG, breathing pattern, and muscle activity maximize information about different stages of arousal or sleep, as well as paroxysmal phenomena like seizures. Eye movements [EOG channel] are recorded using two surface electrodes, placed diagonally, one centimeter above and below the outer canthus of the left and right eyes. Muscle tone is registered using a surface electrode placed over the submental region (chin EMG). ECG is recorded by two electrodes kept over the right and left side of the anterior chest wall. Another option is to place these electrodes over the right and left arms, in which case they can, in addition to the ECG, also record the limb movements. Extra channels for registering movements (transducers or surface EEG electrodes) may be added, for instance in the presence of clonic movements, tremor, hiccups, etc. Respiration is monitored by a transducer kept over the abdomen or the chest. Cessation of breathing for 6–7 seconds may be normally seen in neonates[9,10] and are considered to be pathological apneas meriting treatment only if they last longer than 10 seconds or occur frequently, or are associated with hemodynamic disturbances (like bradycardia or desaturations). Healthy premature infants may show short apneas as well as episodes of periodic breathing.[11] Other polygraphic variables like nasal airflow (measured using a thermocouple) and oxygen saturation (pulse oximetry), if available, may add important information.

Reactivity

Transient attenuation of the EEG to external stimuli should be noted by the technician. Photic stimulation usually does not elicit photic driving in the term neonate and hence need not be done as a routine. However, in the premature infant, a striking photic driving may sometimes be observed, especially with lower flash frequencies.

Description of normal EEG phenomena

Maturation of EEG in the premature infant

Literature describes EEGs recorded in prematures born as early as 23 weeks. The baby is considered to be premature till 37 weeks CA. EEG maturation is summarized in table 1 and figure 2.

Major characteristics

<u>*Background activity*</u>:

Discontinuity: The hallmark of the premature EEG is discontinuity of the background activity (*tracé discontinu*, TD). This is characterized by periods of relative quiescence (voltage <25 μV, sometimes <10 μV), interrupted by bursts of EEG activity. The interburst interval (the length of discontinuity) shows a significant relationship with CA, with the periods progressively shortening with increasing maturation of the brain and development of cortical folding.[12] In addition, with maturation, the ongoing oscillatory EEG activity during the quiescent periods gradually increases in amplitude (becomes >25 μV by 36–37 weeks) and frequency, ultimately resulting in a continuous EEG trace at term.[13,14] The voltages are dependent on the montage used, and are given here as a rough guide for the EEG interpreter to help in classifying the records.

Spontaneous activity transients (*SATs*)**:** The term SATs has been used to describe the slow EEG activity during the bursts,[15,16] taking into account the similarity seen between this and the EEG activity in newborn animals like mice and rat.[17] Parallel with brain maturation, the amplitude of SATs decreases and their duration increases [Table 1 and Fig 2]. Their morphology becomes increasingly complex, with occurrence of waveforms of different frequencies. They usually disappear by 40–42 weeks. Delta brushes (low-amplitude faster frequencies superimposed on high-amplitude slow waves) are probably components of the SATs.[13,17] SATs in the developing somatosensory cortex are driven by early motor activity and may be important for normal development.[18]

Sleep–wake cycles: Changes in state can be detected as early as 27 weeks, but it is only by about 31 weeks that differentiation of sleep–wake cycles (as detected by conventional short-duration EEG registrations) become well established. Awake EEG and active sleep (AS or rapid eye movement sleep, REM) are similar in morphology, in showing a continuous trace consisting of mixed frequencies, but can be differentiated using polygraphy. REM seen on EOG is the hallmark of AS, while wakefulness shows both rapid and slow eye movements. Both show irregular respiration but the (chin) EMG shows very little or no activity (hypotonia) with occasional brief bursts of activity in AS while it shows continuous activity when the baby is awake. Movement artefacts are more frequently seen in the wake EEG. AS may also show anterior slow dysrhythmia at 36–37 weeks. These are short bursts of delta activity occurring over the frontal regions.[14]

Minor characteristics

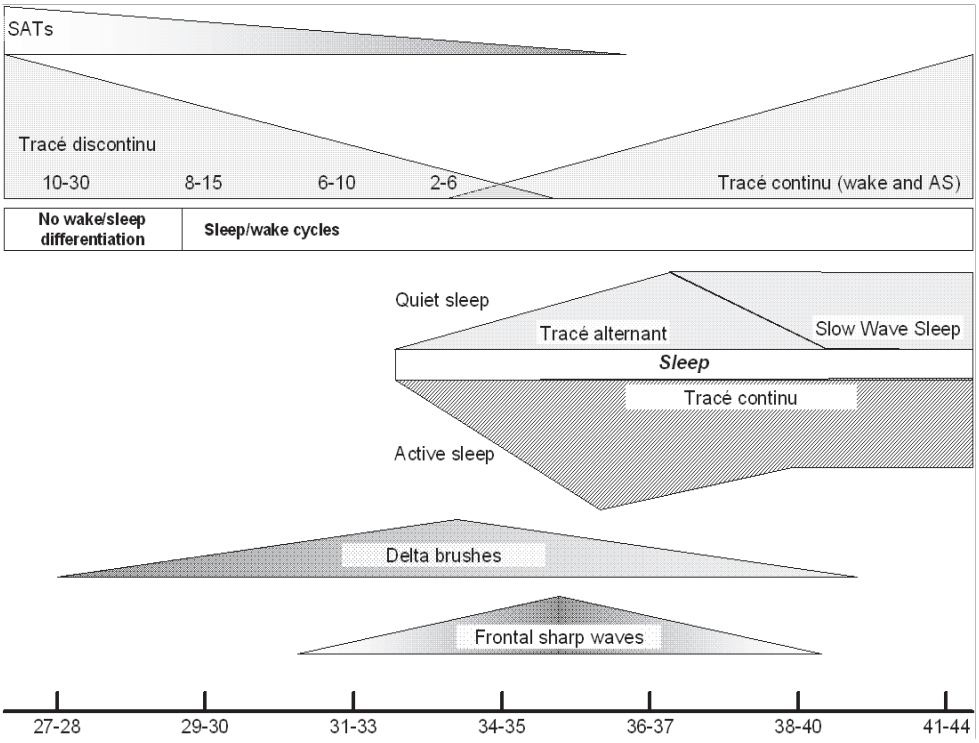
<u>*Paroxysmal waveforms*</u>: These include delta brushes, premature theta in the temporal and occipital regions, biphasic frontal sharp waves (*encoches frontales*), and sporadic sharp waves. Their location and frequency of occurrence varies with CA [Table 1]. Sporadic sharp waves of simple morphology are also seen, at 1–4/minute, varying in their location, more often in the central regions. Frequent sharp waves as well as sharp waves with complex morphology ('polyspikes'), occurring rhythmically or consistently over a focus are considered abnormal. Their association with epileptic seizures is weak, and they are not termed 'epileptiform' at this age. Positive sharp waves in the central[19] and temporal regions[20] are considered abnormal and have been described to have varying association with pathological conditions like intraventricular hemorrhage and periventricular leukomalacia.

Table 1: Salient normal EEG features at different conceptional ages

Age (in weeks)	27–28	29–30	31–33	34–35	36–37	38–40	41–44
EEG feature							
Major characteristics (background activity)							
Tracé discontinu	+++(>80%)	++	+ (50%)	+/-	-	-	-
(discontinuity, sec)	10–30	8–15	6–10	2–6			
SATs high amp	+++	++	+	+	+/-	-	NA
Complexity	+/-	+	+	++	+++	+++	
Sleep–wake cycles	-	+/-	+	++ AS 60%	+++	+++ AS 50%	+++
Tracé alternant	-	-	-	+	+	++	+
Tracé continu Occurrence	-	-	-	+ (W, AS)	+ (W, AS)	+ (W,AS,SQS)	++ (W,S,SQS)
Synchrony (of EEG 'bursts')	++ >80%	+/- ≤50%	+ >50%	+ ≥70%	++ >80%	++ ~100%	+++ 100%
Minor characteristics (paroxysmal wave forms)							
Delta brushes	+/-	++	+++	++	++	+	+/-
Location		C	TO	TO	TO	O	
Occurrence			W, AS,QS	W, AS,QS	QS	QS	
Theta activity	+	++	++	+	+/-	-	-
Location, predom	O	T,O	T,O	T,O			
Delta activity	-	+(AS)	+(AS,QS)	+(QS)	+(QS)	+(QS)	+(QS)
Location, predom		O	O,F	O,F	F	F	F
Frontal sharp waves	-	-	+	++	+	+/-	-
Sporadic sharp waves	+/-	+	+	+	+	+	+/-

Tracé discontinu: Quiet periods show a voltage <25 μV (often < 10 μV); *SATs*: spontaneous activity transients, intermittent slow transients seen in prematures; high amp: higher amplitude (>100 μV), complexity: consisting of waves of multiple frequencies; *Quiet sleep (QS)*: consists of TA and SQS; *Tracé alternant(TA)*, part of *QS*: Quiet periods of ≥25 μV, alternating with bursts of 100–200 μV; *Active sleep(AS or REM)*: differentiated from wakefulness (*W*) by absent muscle tone in chin EMG channel, rapid irregular eye movements in EOG channel, and irregular respiration. *AS* decreases with maturation; *Tracé continu*: Irregular delta and theta of 50–100 μV during wakefulness (*W*) and *AS*. High amplitude (>100 μV) delta during slow quiet sleep (*SQS*); *Delta brushes*: rhythmic beta activity superimposed on delta waves. Amplitude decreases with maturation; *Theta activity*: temporal and occipital saw tooth waves, 4–7 Hz, 0.5–1.5 seconds in duration; *Predom*: predominant; *C*: central; *O*: occipital; *T*: temporal; *F*: frontal; *Frontal sharp waves*: *encoches frontales*; *Sharp waves*: occur sporadically in different locations, predominantly central, 1–4/minute

Fig 2. Scheme illustrating important maturational changes in the neonatal EEG.
Tracé discontinu: duration of discontinuity is given in seconds. At the bottom, conceptional age in weeks



EEG in the healthy term neonate

Awake EEG: Consists of continuous mixed frequency activity (*activité moyenne*) comprised of theta and delta activity of 70–100 µV [Fig 3], with the slower frequencies having a preponderance over the posterior head regions.[14] Short runs of theta activity of 1–3 seconds, and about 50 µV amplitude, are seen over the central region. The faster rhythms (4–8 Hz) also tend to dominate over the frontocentral regions.[21]

Sleep EEG: Sleep periods often start with AS and it constitutes about 50% of the total sleep time. The EEG in AS shows continuous activity in all frequency bands with an amplitude of 50–70 µV. It is similar in morphology to the wake EEG and differentiation is made only with information from polygraphy.

Two types of quiet sleep (QS) are identified: *tracé alternan*t (TA), an alternating pattern of 'bursts' and relatively quiet periods [Fig 4]) and slow QS.[14] TA is reminiscent of the discontinuous EEG of the premature. However, it shows activity higher than 25 µV (usually 40–50 µV) during the quiet periods. The 'burst' periods show theta and delta activity of up to 200 µV. Both periods last for 3–10 seconds. Slow QS consists of continuous high-amplitude delta activity over all regions. Frontal sharp transients (*encoches frontales*) are seen more during QS than AS.[22] Respiratory movements are regular in QS. However, using the above information, the sleep stage cannot readily be classified in about 20% of the situations, and may be termed as 'transitional sleep' or 'indeterminate sleep'.[3]

Fig 3. Awake EEG at term. Shows theta and delta activity with normal voltage (20–70 μV). The faster frequencies (>50 Hz) seen in the temporal channels are due to muscle artefacts. Chin EMG shows rhythmic artefacts produced by sucking. At the top, aEEG trend for a period of five hours. The band width between 5 and 20 μV is normal, and the fluctuations suggest sleep–wake cycling. The 12-second raw EEG sample in the lower part corresponds to the vertical bar on the right of the aEEG

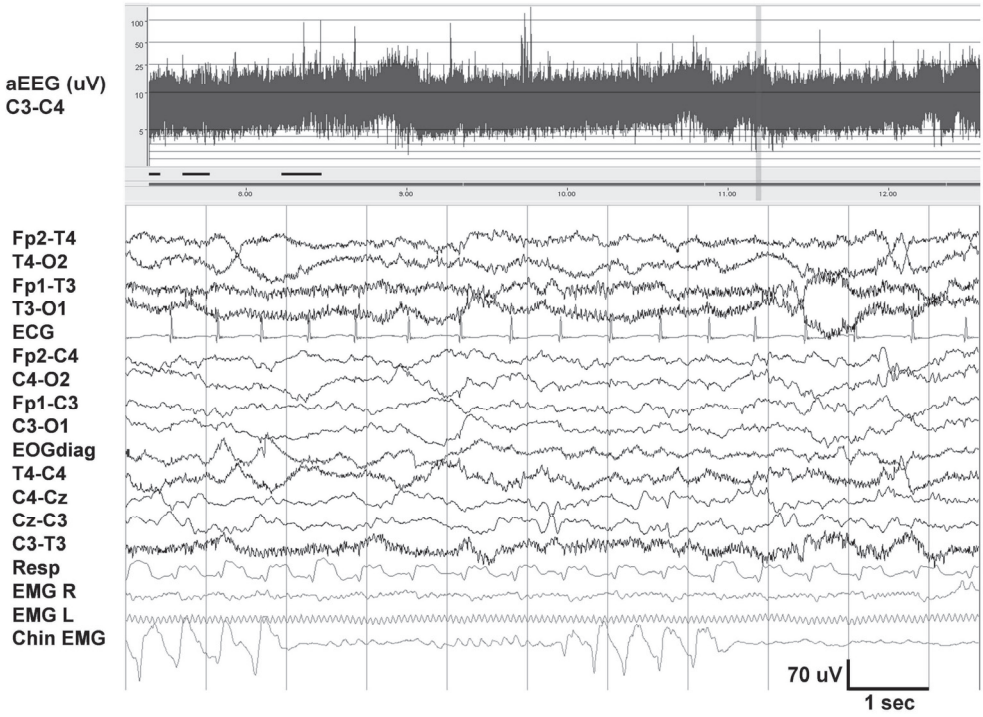
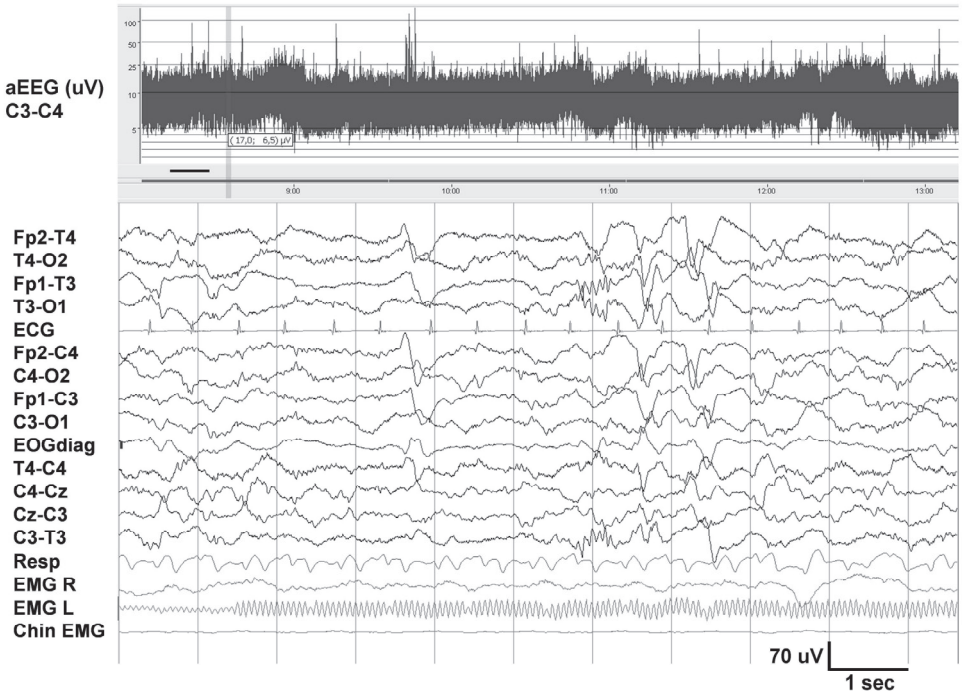


Fig 4. Normal quiet sleep (same neonate as in figure 3). *Tracé alternant* with lower voltage during 2–5 seconds, and higher voltage in the next 3 seconds. Frontal sharp waves (*encoches frontales*) are seen at 4 seconds and brush-like activity between 6 and 7 seconds, over T3-O1. Compared to **Fig 3**, amplitude is higher and there is less muscle activity



Abnormal neonatal EEG activity and its interpretation

Abnormalities in the neonatal EEG background activity and occurrence of excessive paroxysmal activity (both transients as well as epileptiform abnormalities) are seen in many encephalopathies like hypoxic ischemic encephalopathy (HIE).

Abnormalities in background activity

Sleep–wake cycling: One of the earliest abnormalities may be an abnormal or absent sleep–wake cycling.[5] This can be readily detected by trends like single channel amplitude integrated EEG (aEEG).[6] Even in relatively healthy neonates, sleep–wake cycles may be disturbed due to effect of medications or disturbances in the external environment as is often found in the NICU. Conversely, the presence of sleep–wake cycles in the neonatal EEG is a good prognostic sign.

Discontinuity and other abnormalities of voltage: Abnormalities of voltage, seen in encephalopathic neonates, in increasing order of severity are: a) discontinuous EEG [Fig 5], b) continuous low-voltage EEG (voltage < 10 μV throughout the record), c) burst suppression pattern (BS) [Figure 6], and d) an isoelectric EEG or a 'flat trace' (voltage consistently < 5 μV).

Fig 5. *Tracé discontinu* in a neonate with moderately severe HIE. Background activity is of low voltage (<20 μV). The discontinuous periods, with a mean voltage of 10 μV, are interrupted by bursts of EEG activity of 2–3 seconds duration. aEEG trend covering 30 minutes shows a band width of 2.5–7 μV. Sleep–wake cycling is absent.

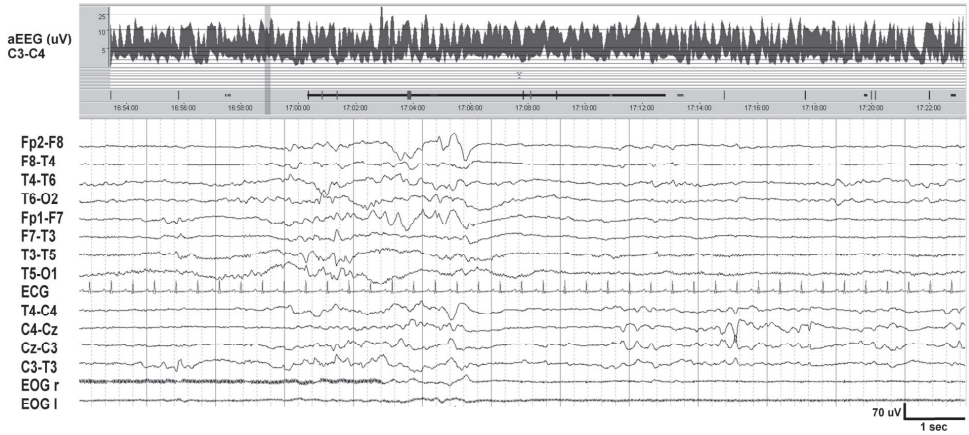
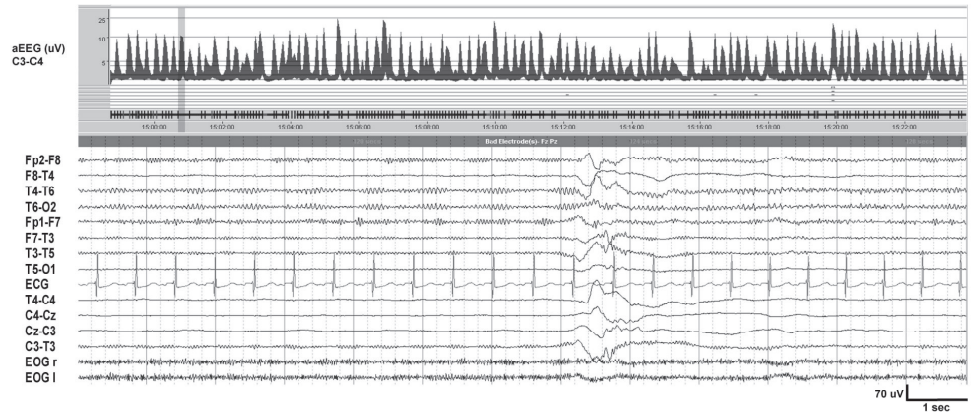


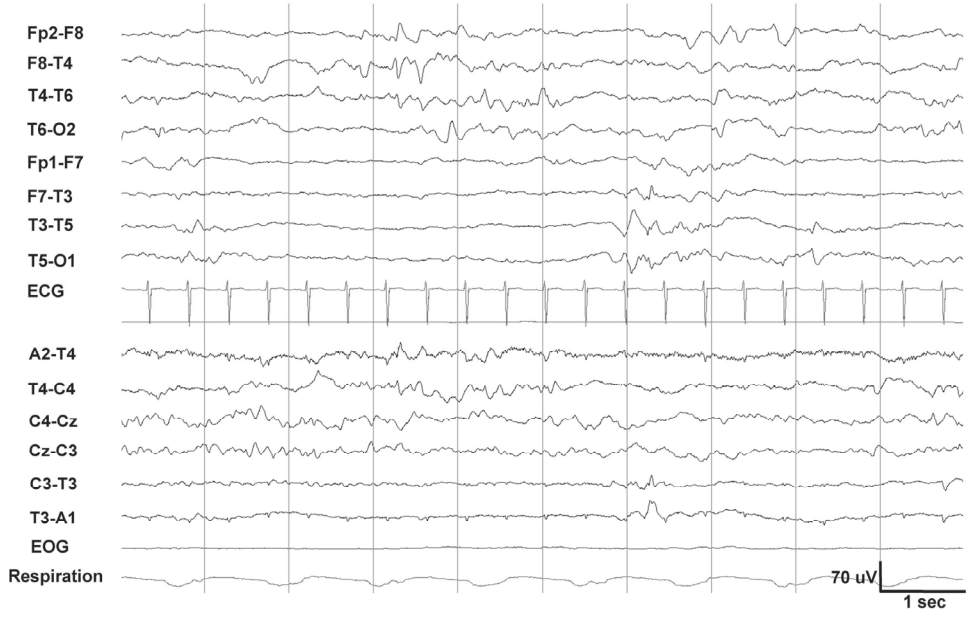
Fig 6. Burst suppression (BS) pattern in severe birth asphyxia. Severely suppressed background activity (<5 µV) with intermittent bursts of 10–20 µV. As opposed to *tracé discontinu* [Figure 5], no EEG activity is discernible during the discontinuous periods. aEEG trend of 30 minutes shows BS and absent variability. Mean band width of the suppressed periods is <2 µV



The discontinuous EEG seen in neonatal encephalopathies like HIE is comparable in morphology to the *tracé discontinu* seen in prematures. The discontinuous periods tend to be longer[23] and of lower voltage (<10 µV) with increasing severity of brain dysfunction and indicate a poor prognosis.[24-27] BS is the severest form of EEG discontinuity and shows prolonged periods (usually >10 seconds) of marked suppression (voltage consistently <5 µV), interspersed with shorter periods of paroxysmal burst activity and a fairly constant interburst interval (absence of variability). The voltage of the bursts may vary and is generally higher than 50 µV. The content of the bursts is also abnormal, with sharper waveforms and less low-frequency activity.[28] The EEG does not show any reactivity to external stimuli.[29,30] Differentiating severe discontinuity from BS can sometimes be difficult,[23] especially in the premature. The EEG hallmark of severe encephalopathies in older children or adults, that is, diffuse theta/delta activity, is typically not seen in the neonate.

Asymmetry: Consistent asymmetry in amplitude of >50% between homologous areas of the brain is abnormal.[24] Etiologies include stroke, sinus thrombosis, hemorrhage, and abscess. The abnormal side usually has lower amplitude with often some of the constituents of the normal background activity missing [Fig 7]. This has to be distinguished from a depression of amplitude with preserved background activity, due to technical reasons (shorter interelectrode distances), edema of the scalp, or a subdural collection.

Fig 7. Neonate with a left middle cerebral artery territory infarct. Voltage asymmetry (suppression of EEG activity) over the left temporal region [see also **Fig 8**]



Asynchrony: Persistent asynchrony (bursts of EEG activity occurring >1.5 seconds apart) between homologous areas of both hemispheres, with <25% of the EEG bursts being synchronous, has been described to be associated with a poor outcome.[24]

Effect of medication: Sedative medications like benzodiazepines and barbiturates transiently affect the background activity in a healthy newborn. However, in a baby with encephalopathy, the effects of these medications on the EEG may be more prolonged, producing attenuation in the voltage of the background activity and producing a discontinuous trace, lasting for up to two hours.[31] When the blood levels of these medications are close to toxic levels, they have a pronounced[32] and sustained effect on the EEG.

Paroxysmal activity: Seizures are a common sign of cerebral dysfunction in neonates[33] and are sometimes the only manifestation. A seizure is recognized as a clear cut change in activity from the ongoing background EEG, with repetitive patterns (sharp waves or rhythmic oscillations in delta, theta, or alpha frequencies) lasting for 6–10 seconds. Often, there is a build up of activity with change in amplitude and frequency [Figures 8 & 9]. There is no consensus regarding the length of these discharges.[34] The term brief rhythmic discharges have been used to describe discharges that are shorter than 10 seconds and these probably have the same clinical significance as seizures.[35] The causes of seizures include HIE, metabolic disturbances, intracranial hemorrhages and infarcts, intracranial infection, and developmental anomalies. There is controversy about whether they are just epiphenomena or cause additional brain damage. An increasing number of studies have shown that neonatal seizures are associated with lasting changes in the CNS[36,37] and are related to poor outcome[38-41]. They are further discussed below.

Fig 8. (Same patient as in **Fig 7**) An electrographic seizure over the left temporal region. A frequency of 5 Hz is seen initially. The amplitude then increases, the morphology becomes more complex, and subsequently, the frequency slows down to 3 Hz. This type of evolution is fairly typical for neonatal seizures.

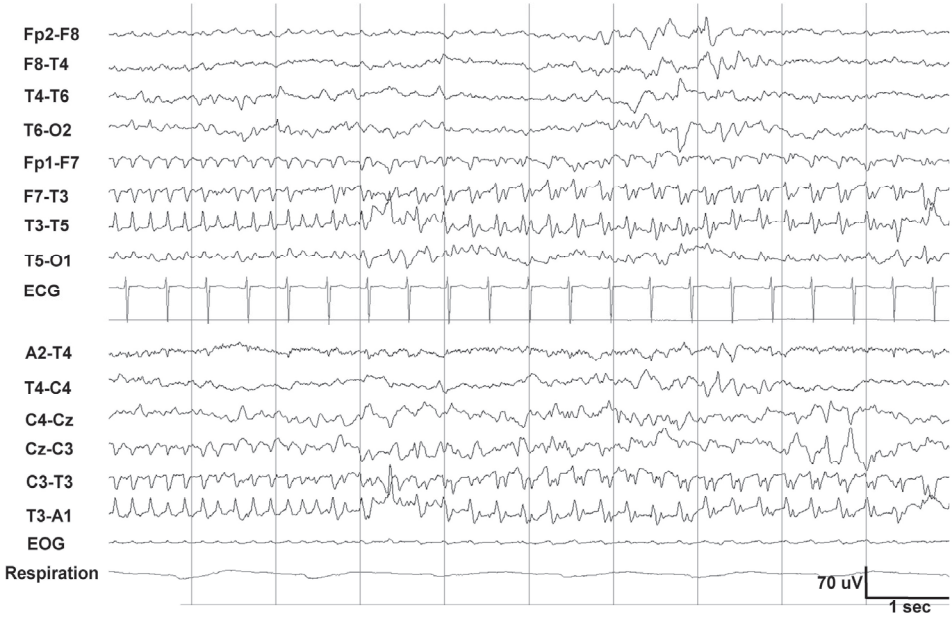
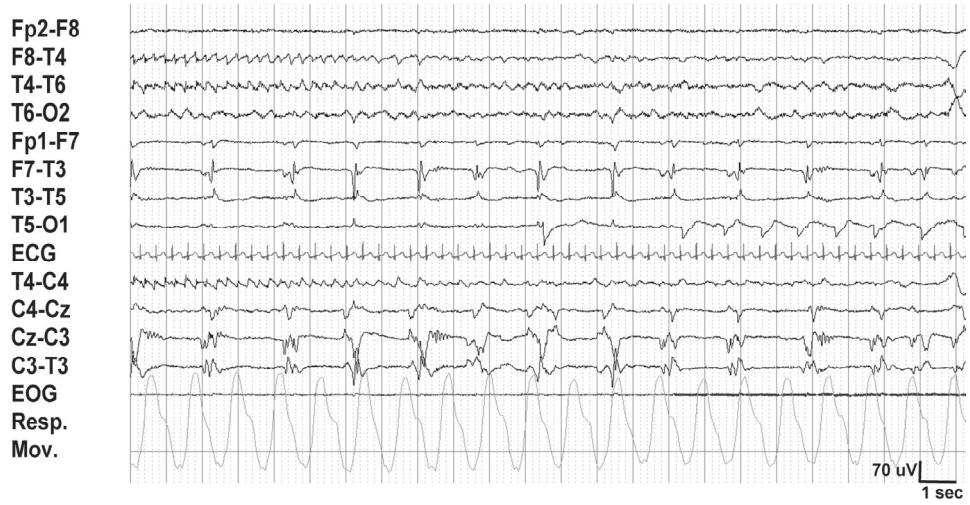


Fig 9. Multifocal seizures on a severely suppressed EEG background in severe birth asphyxia, indicates poor prognosis. The neonate died.



EEG monitoring in the neonate

EEG monitoring is indicated in neonates with moderate to severe encephalopathy.

Selection of patients for monitoring

At our center, neonates with the following clinical indications are taken up for continuous monitoring with EEG polygraphy (including video).

1. Neonates with features of severe birth asphyxia: Apgar score ≤5 at 5 minutes, umbilical cord blood pH <7.1.

2. Babies with or without asphyxia but strong clinical suspicion of seizures, like repetitive rhythmic limb movements or unexplained apneas.

3. Neonates with encephalopathy (Sarnat[42] score 2–3), regardless of severity of asphyxia.

4. Critically ill neonates on ventilator, especially if neuromuscular blocking agents are used.

5. If a standard EEG shows moderate to severe abnormalities in the background activity (discontinuity, persistent asymmetry, or presence of epileptiform abnormalities).

If the initial EEG shows a normal or mildly abnormal background activity, continuous EEG monitoring gives little extra information, unless the clinical condition of the neonate subsequently deteriorates or there is suspicion of seizures.

Aims of monitoring

a) Assessment of background activity and detection of changes over time, with the goal of early detection of changes, at a stage when brain dysfunction is potentially reversible. The information can also be used for prognostication.

b) Detection of (subclinical) seizures.

c) Assessment of response to treatment.

Assessment of dynamic changes in background activity (improvement/deterioration): EEG monitoring is invaluable in assessing the degree and reversibility of brain damage in HIE. Severely abnormal EEG background activity is associated with poor neurological outcome. Persistence of abnormal patterns for 24 hours or more, or worsening of background activity denote poor prognosis.[26,43] However, improvement of the background activity (increasing voltage, decrease in discontinuity, appearance of sleep–wake cycles) within 12–24 hours after birth is a good prognostic feature. Medications[23] such as morphine, phenobarbitone, midazolam, and lidocaine, as well as metabolic or electrolyte disturbances can produce suppression of background EEG voltage, lasting for minutes to hours.

Detection of seizures: majority of neonatal seizures are subclinical: EEG monitoring increases the chance of detecting paroxysmal abnormalities like seizures. Their detection is based on clinical observation in conjunction with (video) EEG. In neonates, the clinical seizures are often subtle[44] and may be missed without constant supervision.[45] Furthermore, in the sick neonate, majority of seizures are subclinical,[46,47] and can be detected only by EEG monitoring.[48,49] A recent study of 51 term neonates with encephalopathy using video EEG monitoring showed that only 34% of the electrographic seizures had clinical manifestations evident on the simultaneous video recording.[50] Only 9% of all electrographic seizures was accompanied by clinical manifestations that were recognized by neonatal staff.[50] Electroclinical dissociation is also common after treatment with antiepileptic drugs (AEDs).[51] All these emphasize the need for continuous EEG monitoring in the encephalopathic neonate. However, EEG analysis requires particular skills which are not always present around the clock in the NICU. Hence, there is scope for an automated system that reliably detects neonatal seizures.

Many seizure detection algorithms have been described.[52-57] The complex nature of the EEG signal results in false-positive seizure detections, especially in methods which show high sensitivity, like that of Liu *et al*.[52] Alternate methods of seizure detection like motion analysis and [58] heart rate analysis[59] have also been explored and were found to be suboptimal.[59] We

have recently been involved in developing a seizure detection algorithm mimicking a human observer, with very promising results.[60] Both under and over-diagnosis of neonatal seizures are potentially harmful to the neonate.

Clinical utility of EEG monitoring for seizure detection: In an increasingly cost-conscious medical system, judicious use of EEG monitoring can be done by selecting those neonates with higher risk for developing seizures. This selection may be done using either clinical criteria (e.g., using Sarnat scores for grading encephalopathy[42]) or EEG criteria. Abnormalities of background activity in a standard EEG done within 24 hours of birth is also a good indicator of the risk of seizures [Figures 7 and 8].[61] The degree of suspicion should be high and threshold for starting EEG monitoring low, if more neonates having seizures are to be detected. Post-hypoxic seizures usually occur within 24 hours, and may continue for up to 72 hours. In a study of continuous EEG monitoring for neonatal seizures, having more than 25 seizures/day, total daily duration of seizure activity more than 30 minutes, or persistence of seizure activity for more than 48 hours predicted death or major neurological sequelae.[43]

The current practice in most neonatal NICUs doing EEG monitoring is to treat subclinical seizures. However, whether this improves the neurological outcome has not yet been proven. Some data[62] suggest a better outcome for treated neonates.

Assessment of response to treatment:

Seizures respond to first-line AEDs like phenobarbitone and phenytoin in less than half of the patients.[63] Midazolam is the usual second-line AED. Seizures resistant to these may respond to lidocaine. Monitoring helps to determine the response to treatment. In some patients there is a period of initial response[64] followed by recurrence of seizures despite maintaining therapeutic levels of the AED. Seizures that are resistant to multiple AEDs usually suggest a poor neurological outcome.[65]

Length of EEG monitoring

In the encephalopathic neonate, the yield is maximum if monitoring is started as soon as possible. In conditions like HIE, starting within 24 hours of birth is suggested. However, there is no consensus regarding how long the monitoring should be continued. The following are suggested as reasonable endpoints.

1. Abnormal EEG background improves and normalizes (disappearance of discontinuity, improvement in voltage, return of sleep–wake cycles)
2. Persistently abnormal EEG background even after 24 hours, if other reversible causes like metabolic disturbances have been excluded. This suggests a poor prognosis.
3. No electrographic seizures for at least 12 hours (if monitoring was started for seizures).

Use of EEG trends for monitoring

Long-term EEG monitoring is labor-intensive. Even in centers where neonatal EEG monitoring is available, around-the-clock support by the clinical neurophysiologist is not possible. This has lead to the development of EEG trends displaying compressed data. Different parameters like aEEG, total power, spectral edge frequency, and entropy have been studied and many of them are included in commercially available EEG equipments. Among these, aEEG has had good acceptance in neonatal ICUs. The following discussion is about the uses and limitations of this technique.

Amplitude integrated EEG

aEEG (Cerebral Function Monitor, CFM™) displays a simple trend, of the peak-to-peak amplitude derived from a single channel (P3-P4) of EEG. The signal is filtered (band pass 2–15 Hz), rectified, and displayed after compression, with a time base of 6 cm/hour (about 5 hours of EEG data displayed per page). A semi-logarithmic scale is used, that is, EEG activity of lower amplitudes (0–10 μV) which is of maximal interest to the clinician, is displayed on a linear scale while the higher amplitudes (10–100 μV) appear more compressed (logarithmic scale). In the healthy neonate, the aEEG band runs between 10 and 20 μV, with fluctuations in voltage reflecting changes in state. Sleep–wake cycling can readily be recognized [Figures 3 and 4].

Background activity

Al Naqeeb *et al*,[66] classified the aEEG background into three groups in the full-term neonate using different voltage cut-offs for the median upper (UM) and lower margins (LM): Normal (UM > 10 μV, LM > 5 μV), moderately abnormal (UM > 10 μV, LM < 5 μV), and suppressed (UM < 10 μV, LM < 5 μV). Another classification of aEEG in the term neonate, based more on pattern recognition than absolute amplitudes, recognizes five major patterns:[67-70] continuous normal voltage (CNV, band 25–10 μV), discontinuous normal voltage (DNV, UM > 10 μV, LM < 5 μV), continuous low voltage (CLV, band $\leq$ 5 μV), burst suppression (BS), and flat trace (FT, UM < 5 μV). This classification is more detailed, and more studies have been published based on this. In term neonates with postasphyxial HIE, the CNV and DNV at six hours correlated with a good outcome while CLV, BS, and FT predicted poor outcome.[68] Classification of background activity by aEEG was shown to have a good agreement with raw EEG.[71]

Detection of seizures

aEEG has moderate sensitivity for detecting seizures.[71-73] They are seen as an abrupt increase in voltage of the upper and lower margins of the trace, along with a narrowing of the band. Seizures in the centroparietal region are readily detected. Different seizure patterns described by aEEG include single seizure, repetitive seizures, and status epilepticus (the so-called saw-tooth pattern).[71]

Advantages and limitations of aEEG

aEEG scores over full-channel raw EEG by its ease of registration and interpretation and is suited for long-term monitoring by the bedside. It is ideal for observing long-term trends in the brain functions like improvement or deterioration of background activity voltage, effect of medications, occurrence of seizures, etc.[69,74]

However, due to the use of only a single EEG channel, important spatial information is lost. Thus, asymmetries as well as seizure activity may be missed. The data compression sometimes results in incorrect estimation of the background activity voltage and missing of seizures that are very focal, of short duration, or of low voltage. Also, contamination by artefacts like ECG, muscle activity, or high-frequency ventilation may result in falsely elevated aEEG voltage and sometimes lead to false-positive 'seizure detection'.[75,76]

Many aEEG monitors also display corresponding single channel of raw EEG, and this helps in improving the quality of interpretation, like recognizing artefacts and confirming occurrence of paroxysmal activity like seizures. aEEG is best viewed as being complimentary to standard EEG. Whenever major therapeutic decisions have to be taken (e.g., when recurrent seizures are seen and an AED infusion is being considered), it is advisable to review the corresponding raw EEG data if available, or record a standard EEG.

Estimating prognosis using EEG

Both EEG and aEEG background activity has been correlated well with neurological outcome. A normal background activity is strongly correlated with good outcome in term neonates. Also, a severely abnormal background activity (persistent low voltage, inactive record, as well as an unvarying BS pattern) is correlated with poor outcome.[77] The background patterns occurring between these two extremes are more difficult to use in outcome predictions, with different studies giving varying results. In such situations, clinical assessment coupled with serial EEGs or EEG monitoring, as well as multimodal evaluation using neuroimaging (ultrasonogram, MRI), and evoked potentials (SEPs, VEPs)[78,79] would help in prognostication. Persistence of the abnormal patterns in the EEG has more prognostic

value.[80] Assessing the prognosis in prematures is more difficult as co-morbidities like pulmonary problems may determine the outcome.

Neonatal epilepsy

Though most neonatal seizures are acute symptomatic (provoked) in nature, a few epilepsy syndromes can manifest in this period. Some are benign while others have a grave prognosis. It is beyond the scope of this article to describe various neonatal epilepsy syndromes. However, some of these rare syndromes with characteristic EEG features are described below as it is important to differentiate them from acute symptomatic seizures. Majority of these have a poor neurological outcome.

1. *Pyridoxine dependency*: Presents with myoclonic and tonic-clonic seizures with or without an associated encephalopathy. EEG findings include generalized high amplitude slowing of the background activity, multifocal or generalized epileptiform abnormalities, or in severe cases, a BS pattern.[81] A high degree of clinical suspicion is needed to diagnose this condition and early treatment with pyridoxine is indicated.

2. *Herpes simplex encephalitis*: Presents in the neonatal period with fever, seizures, and obtundation. EEG findings include generalized or asymmetric slowing of the background activity, and periodic lateralized epileptiform discharges.[82]

3. *Nonketotic hyperglycinemia* can present in the neonate with refractory seizures and a BS pattern.[83]

4. *Ohtahara syndrome (Early infantile epileptic encephalopathy or EIEE) and early myoclonic encephalopathy (EME)*: Both can show a BS pattern on EEG. The BS (sometimes asymmetric) in EIEE is present in both wakefulness and sleep and persists unchanged for about two weeks, while in EME, it is seen mainly in sleep.[84] EIEE is caused by cortical malformations or migrational disorders, and presents with refractory tonic seizures while EME is caused by inborn errors of metabolism and usually presents with myoclonic seizures.

Summary

Neonatal EEG gives important information about brain maturation and function and complements clinical examination. It also helps in detecting seizures and in prognostication.

- The technician should note the CA and clinical setting of EEG registration.
- Polygraphy helps in differentiating awake and sleep states and in recognizing artefacts.
- EEG is classified as normal/abnormal for CA. Reviewing at a time base of 20 seconds/page makes detection of slow activity and periodic or rhythmic phenomena easier. Abnormalities are classified as mild, moderate, and severe.
- Mild abnormalities include dysmaturity, excessive sporadic sharp transients, etc.
- Moderate abnormalities include abnormal or absent sleep–wake cycles, excessive discontinuity, persistent asymmetry, and epileptiform abnormalities including seizures.
- Severe abnormalities include persistent low voltage, BS, and inactive/isoelectric EEG.
- When moderate or severe EEG abnormalities are detected, EEG monitoring can give additional information regarding the evolution of background activity and the occurrence of seizures.
- EEG trends like aEEG are useful for the bedside monitoring of sick neonates, provided its benefits and limitations are well understood.

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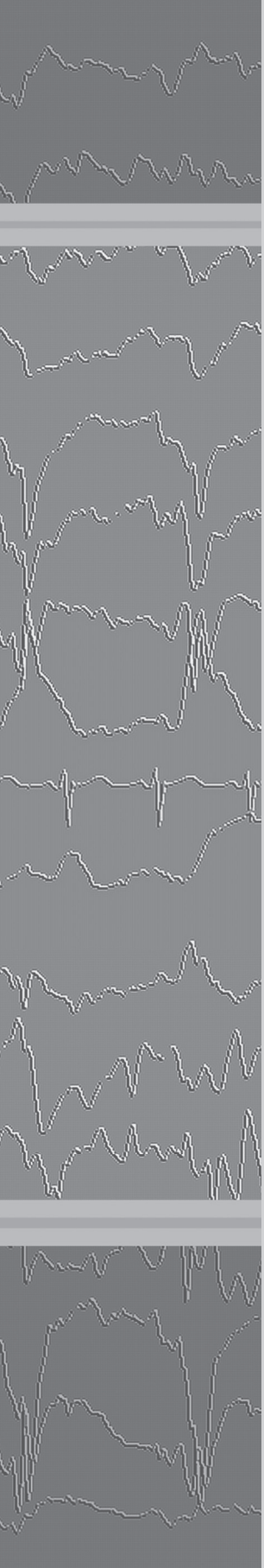

Chapter 2

Relationship of neonatal seizure characteristics to brain injury

2.1 EEG characteristics of neonatal seizures relate strongly to the severity of hypoxic brain injury and outcome

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ABSTRACT

Aim: To relate electrographic seizure characteristics, EEG background abnormalities, and outcome in perinatal asphyxia.

Methods: EEG background and seizure characteristics were studied in 42 consecutive term neonates with post-asphyxial seizures using video-EEG polygraphy for ≥24 hours, starting <24h post partum. An eight-grade EEG background score was developed. For statistical analyses, patients were grouped into those with mild to moderate (group I, n=20) and severe (group II, n=22) abnormalities of EEG background. Brain MRI was available in all except three infants. Survivors were followed for at least two years.

Results: There was a positive correlation between EEG background abnormality and total seizure number (*rho* 0.73, $p<0.001$) and total seizure burden in minutes (*rho* 0.41, $p<0.01$). In contrast, mean individual seizure duration (*rho* −0.51, $p<0.01$) and amplitude (*rho* −0.44, $p=0.004$) were negatively correlated with background. Significantly more patients in group II had seizures that spread to the contralateral hemisphere, seizures with a dominant frequency >3 Hz, and >50% of arrhythmic seizures, as well as lesions of basal ganglia and thalami on MRI. There was a moderate correlation between poor outcome (defined as death or severe handicap) and total number of seizures per patient (*rho* 0.61, $p<0.001$) as well as seizure burden (*rho* 0.39, $p=0.01$). Logistic regression analysis showed that EEG background strongly predicted ($p=0.003$) poor outcome. Neither total seizure number, burden, nor MRI confirmation of lesions of basal ganglia and thalami significantly added to this. For patients in EEG group I, there was a trend towards significance for total seizure burden (*rho* 0.46, $p=0.055$) to be associated with poor outcome.

Conclusion: Electrographic characteristics of neonatal seizures were strongly associated with the severity of underlying hypoxic brain injury as shown by EEG background abnormality, which in turn was the strongest predictor of poor outcome. In this cohort with relatively more patients with severe hypoxic encephalopathy, seizures were not independently associated with poor outcome. This implies that in future studies of treatment and outcome of persistent post-asphyxial subclinical seizures, stratification of neonates according to severity of EEG background abnormalities is indicated.

INTRODUCTION

Neonatal seizures frequently occur in the context of hypoxic ischemic encephalopathy (HIE)[1]. HIE causes both cortical and subcortical (white matter, deep grey matter and brainstem) injury, while seizures predominantly reflect cortical functional disturbances. EEG monitoring can be used to assess the severity of HIE by studying the evolution of EEG background abnormality[2,3], as well as to study electrographic characteristics of seizures [4,5]. Both seizures [6] and EEG background abnormalities [4,7-9] are associated with poor outcome. However, it is difficult to assess the independent contribution of each factor to outcome, primarily because an abnormal background activity[6,10] or severe injury on histopathological examination[11,12] is strongly associated with electrographic and clinical seizures.

The above implies that the independent and interactive effects of seizures and HIE on outcome may be addressed through an EEG study that captures adequately both seizure and background activity. Such a study has not yet been performed. Two studies from the same research group found that a composite 'seizure score' (which includes among others, parameters such as interictal sharp waves and abnormality of background activity seen on a standard EEG), correlates with both brain injury as detected by H^1MRS[13] as well as with clinical outcome[14], independent of 'structural brain injury' as detected by MRI. Their score is better termed as an 'encephalopathy plus seizure score'. Moreover, no EEG monitoring was performed and hence the authors could derive no accurate measures of seizure burden. It is well known that clinical observation alone fails to detect neonatal seizures reliably, as neonates with encephalopathy frequently show electro-clinical dissociation[15].

In a study by McBride et al.[6], continuous EEG monitoring allowed adequate sampling of the electrographic seizures. However, they did not include EEG background as a co-variate in their regression analysis.

Another but somewhat related issue is that it is difficult to predict outcome in HIE when the EEG background is mild to moderately abnormal. We hypothesize that this prediction may be improved through the inclusion of electrographic seizure characteristics that are known to be associated with poor outcome, such as measures of seizure burden [5,13], number of seizure foci[4], contralateral spread [16] and persistence for ≥48 hours [5].

This study aims to assess the relationships between post-asphyxial electrographic seizure characteristics, EEG background, and clinical outcome through detailed analysis of long-term EEG registrations. Secondary aim is to determine whether there is additional value in studying seizure characteristics like number of foci, frequency, rhythmicity and spread,

especially in neonates with mild to moderate EEG background abnormalities. With this contribution we hope to refine prognostication and also to better guide clinicians during treatment of post-asphyxial seizures.

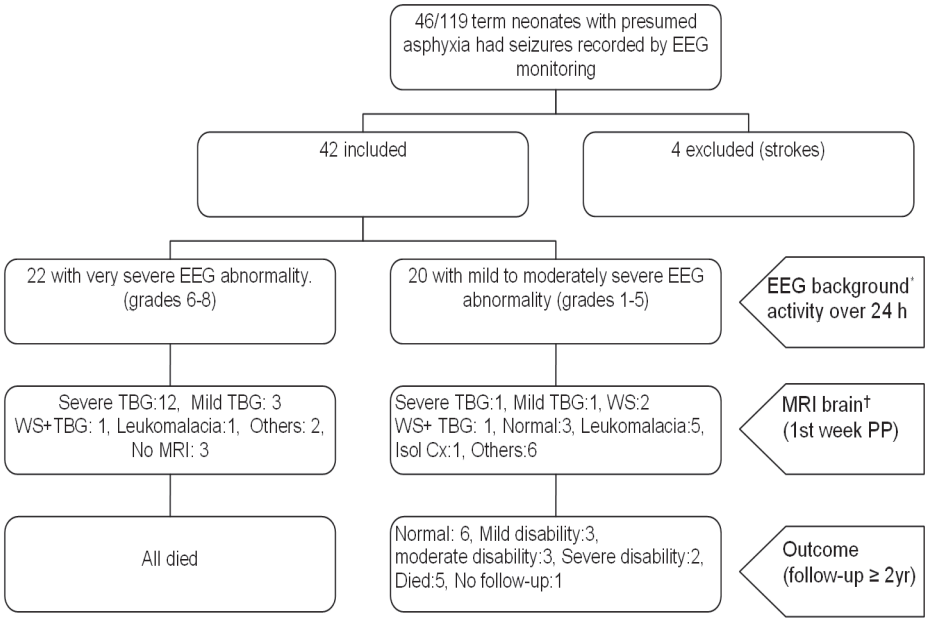
PATIENTS AND METHODS

Patients

EEG registrations were performed in 119 consecutive term newborns with presumed perinatal asphyxia. All underwent video-EEG monitoring for ≥24 hours (h), between March 2003 and August 2007 as part of an ongoing study (Fig 1). The inclusion criteria for EEG monitoring were: gestational age of 37-43 weeks with clinical features of encephalopathy, and having at least one of the following features of birth asphyxia: a) arterial pH of umbilical cord blood ≤7.1, b) Apgar score ≤5 at 5 minutes, and c) high clinical suspicion (like fetal distress, umbilical cord prolapse, difficult labour, or a history of convulsions). The inclusion criteria for asphyxia were deliberately kept broad so as not to miss patients with post-asphyxial seizures. Babies with congenital cardiac abnormalities, multiple congenital anomalies and inborn errors of metabolism were excluded. None were treated with hypothermia. The study had the approval of the Erasmus MC Medical Ethical Review Board. Informed consent was obtained from the parents/guardians prior to the onset of the registration.

Electrographic seizures were present in 46 babies. Of these 46, four neonates with ischemic stroke involving well-defined arterial territories on MRI (suggesting a non-asphyxic etiology of the seizures) were excluded. Data from the remaining 42 were used for analysis. All received loading doses of phenobarbitone (20 mg/kg) and subsequently, due to persistent electrographic seizures, intravenous midazolam was given as a loading dose of 0.1 mg/kg, followed by maintenance doses of 0.1–0.5 mg/kg/h. Seventeen out of the 42 babies with seizures refractory to midazolam received intravenous lidocaine, 2 mg/kg, followed by a maintenance dose of 6 mg/kg/hour, which was weaned off over 30 h. The antiepileptic drug (AED) treatment followed the departmental protocol, with every next step taken when no effect on electrographic seizures was seen within one to two hours. This protocol is similar to the ones used in other European neonatal intensive care units [17].

Fig 1. Inclusion and follow-up of patients with electrographic seizures. *h*: hours, *PP*: post partum, *TBG*: Thalami-Basal Ganglia injury, *WS*: Watershed, *Isol Cx*: isolated cortical injury, *yr*: years. Odds Ratio (95% CI) for severely abnormal outcome for patients with [*]mild to moderately severe EEG background (grades 1–5) abnormalities was 0.21 (0.11-0.44) and [†]without TBG injury on MRI was 0.24 (0.12-0.48).



EEG registrations

Digital video-EEG with polygraphy, started mostly ≤ 24 h post partum (PP), was registered continuously for 1-3 days using a Nervus™ monitor (Taugagreining hf, Reykjavik, Iceland). Scalp electrodes were applied according to the 10-20 International System as described in more detail elsewhere [18]. In eight patients, we used a restricted 10-20 system using 13 electrodes (Fig 2A). EEG sampling frequency was 256 Hz. Band-pass filter was 0.3–70 Hz. As a part of standard patient care, the EEGs were interpreted by a clinical neurophysiologist at the time of the registration and this information was used to make treatment decisions. All the data were digitally available for review.

EEG background scoring

We developed an eight grade scoring system for the quantification of EEG background abnormalities (***Appendix***) that was partly derived from the literature [7-9,19] and partly based on our clinical experience. This scoring system emphasizes discontinuity (voltage and length of the discontinuous periods) and its evolution over 24 h, and includes the presence or absence of variability, reactivity, and sleep-wake cycles (SWC). We omitted parameters such as frequency content and duration of the EEG bursts, and frequency of sporadic or rhythmic sharp waves within and outside the bursts, because we wanted the classification to be readily applicable at the bedside.

The severity of discontinuity is fairly easy to assess and has been shown to be a robust parameter in the prediction of outcome [3]. Discontinuity was defined as voltage attenuation (< 25 μV for mild and <10 μV for severe discontinuity) in at least 50% of the EEG channels, lasting for ≥5 seconds. For all EEG segments that qualified as discontinuous according to these criteria were quantified, the amplitude and duration were scored. As overall discontinuity score, the most frequent measures of the amplitude and duration of the discontinuous periods were used.

For this study, EEGs were scored by an experienced clinical neurophysiologist (PJC) together with a neonatologist (RMS), blinded to the clinical and MRI results. Consensus was reached in all cases. Since the number of patients was insufficient for a three-way classification (mild, moderate, and severe abnormalities) to be used in the statistical analyses, patients were re-classified into two groups, with group I presenting normal to moderately severe grades (0-5) and group II severely abnormal grades (6-8).

Electrographic seizures

Electrographic seizures were defined as clear variations from the background activity, displaying a repetitive pattern of oscillations (sinusoidal waves) or sharp waves or a mixture of both, lasting ≥10 seconds, with evolution in amplitude and frequency over time [20-22]. The inter-rater agreement among two clinical neurophysiologists (PJC and GHV) for the scoring of seizure occurrence in one hour segments of EEGs from 10 patients has previously been established to be 73%, with a *Kappa* value of 0.4 [22].

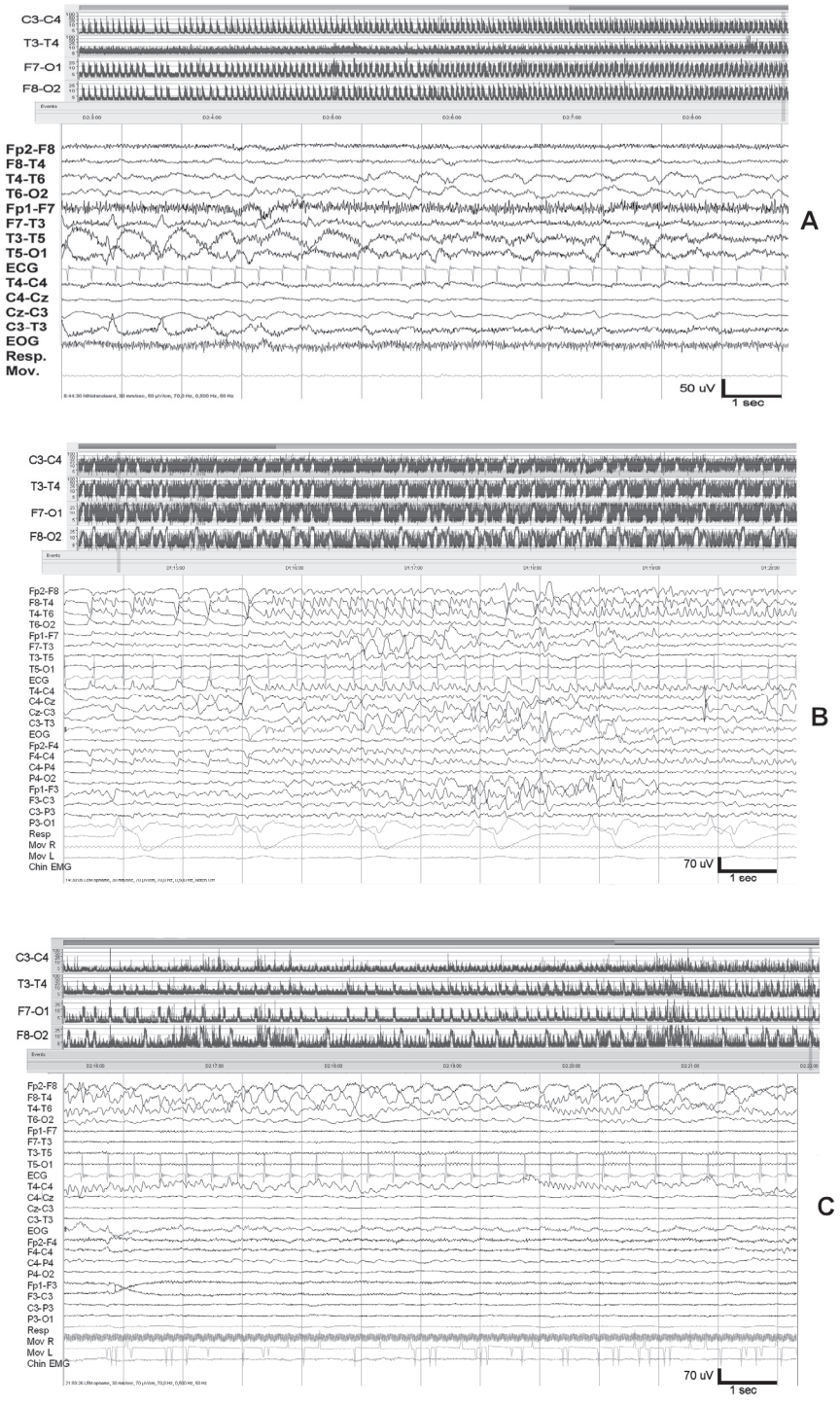
For the purpose of this study, all recordings were reviewed by a clinical neurophysiologist (PJC) and the seizures were scored for their onset, frequency, amplitude, duration,

rhythmicity, location and spread. For each patient, the total number of seizures as well as the cumulative duration of all epochs of seizures in minutes (total seizure burden) was determined. Unfortunately, there is no consensus about the best way to quantify seizure burden during long periods of EEG monitoring. A major problem is that the extent to which seizures are expressed varies with time PP, with seizure onset usually occurring within 24-48 h PP. This implies that the ictal fraction [cumulative duration of all seizure epochs divided by total duration of monitoring [16]] is sensitive to the time of onset PP of the recordings and to the duration of the monitoring. As an attempt at standardization, we therefore also measured the total seizure number and ictal fraction expressed by each patient in the 24 h period during which the most seizure activity was recorded.

The other ictal parameters were defined as follows:

- Contralateral spread: an electrographic seizure that develops over contralateral homologous areas during the seizure or within 5 seconds of termination of the initial seizure discharge, expressing similar morphology and frequency, but evolving independently [23] (Fig 2A).
- Fast seizure: electrographic discharge expressing a dominant frequency of >3 Hz (disregarding the usually slower onset of ≤2 Hz), lasting ≥10 seconds (Fig 2B). Seizures that showed a short burst of fast activity (5–14 Hz) lasting for 3 to 5 seconds followed by a slower dominant frequency of 0.5-2 Hz were not deemed "fast".
- Arrhythmic seizure: marked variability in the interval between individual complexes in a seizure discharge for the major part of its duration (Fig 2C). Even though it is possible to mathematically define rhythmicity [22], we kept this as a parameter that is visually scored. We considered it significant only if >50% of seizures in a patient were arrhythmic.

Fig 2. ***Electrographic seizure characteristics (see next page)*****:**
The vertical grey line over the aEEG trends corresponds to the EEG page displayed.
A. Spread of a left temporal-central (T5-C3) seizure to contralateral hemisphere (to right temporal,T6). EEG background grade 8. The aEEG trend shows a saw-tooth pattern suggestive of status epilepticus.
B. A fast (>3Hz) seizure over the right temporal region. EEG background grade 3
C. An arrhythmic seizure over the right temporal region. EEG background grade 8.


C3-C4
T3-T4
F7-O1
F8-O2
Fp2-F8
F8-T4
T4-T6
T6-O2
Fp1-F7
F7-T3
T3-T5
T5-O1
ECG
T4-C4
C4-Cz
Cz-C3
C3-T3
EOG
Resp.
Mov.
50 uV
1 sec
A
C3-C4
T3-T4
F7-O1
F8-O2
Fp2-F8
F8-T4
T4-T6
T6-O2
Fp1-F7
F7-T3
T3-T5
T5-O1
ECG
T4-C4
C4-Cz
Cz-C3
C3-T3
EOG
Fp2-F4
F4-C4
C4-P4
P4-O2
Fp1-F3
F3-C3
C3-P3
P3-O1
Resp
Mov R
Mov L
Chin EMG
70 uV
1 sec
B
C3-C4
T3-T4
F7-O1
F8-O2
Fp2-F8
F8-T4
T4-T6
T6-O2
Fp1-F7
F7-T3
T3-T5
T5-O1
ECG
T4-C4
C4-Cz
Cz-C3
C3-T3
EOG
Fp2-F4
F4-C4
C4-P4
P4-O2
Fp1-F3
F3-C3
C3-P3
P3-O1
Resp
Mov R
Mov L
Chin EMG
70 uV
1 sec
C

To study the potential value of adding the above seizure parameters to the background score in order to improve prognostication of moderate HIE, patients in EEG group I (with mild to moderately severe background abnormalities) were further classified based on whether they had poor outcome or not (see below).

Neuroimaging

MRI scans of the brain were performed in the first week after birth (median day 4) in 39 patients according to an asphyxia protocol on a 1.5 Tesla scanner. All scan sessions included conventional T1& T2-weighted spin-echo, and proton density images, as well as diffusion weighted images with apparent diffusion coefficient mapping. Images were classified using information from all sequences, by an experienced pediatric neuroradiologist (ML) who was unaware of the results of the EEGs and outcomes[24]. Because of the small number of patients per brain injury pattern, they were further clustered into two classes, depending on whether there was injury to deep grey matter (thalamus and striatum) or not, which is a very good predictor of neuromotor outcome[25].

Outcome

A pediatrician unaware of the EEG results performed follow-up examinations until at least two years of age, using Bayley Scales of Infant Development (BSID)[26]. Patients with a normal short-term development at one year, who were not further followed at our clinic, were assessed with a telephone questionnaire based on the Pediatric Stroke Outcome Measure [27] administered to the parents. Those in whom cerebral palsy (CP) was detected were referred to the pediatric neurologist. In these patients, the Gross Motor Functional Classification System (GMFCS) [28] was used to classify the severity of CP.

Outcome was initially classified into five categories:

1. normal
2. mild motor disability (no CP) or language/cognitive delay
3. moderate disability, CP (GMFCS 1 or 2)
4. severe disability (GMFCS 3-5 or Mental Developmental Index [BSID MDI] < 70)
5. dead.

For the purpose of statistical analysis, these five groups were combined into two, as good/mild to moderate abnormality (grades 1-3) and poor (grades 4-5) outcomes.

Statistical analysis

Association between two or more groups was studied using Spearman's rank correlation. Comparison of two groups was done using Mann-Whitney U test for skewed data or Fisher's exact test for categorical variables. Logistic regression analyses were done with poor outcome as the dependent variable and grades of EEG background abnormality along with either total seizure number, seizure burden or presence of lesions of basal ganglia and thalamus on MRI as covariates (there were insufficient number of patients to use more than 2 covariates at a time). All tests were performed using SPSS^{TM} version 15.0, unless otherwise specified. A p value of <0.05 was considered significant.

RESULTS

A total of 1841 h of EEG monitoring data was reviewed. The clinical and EEG monitoring characteristics of the patients are given in Table 1, subdivided for the groups of patients with mild to moderately abnormal background activity (n=20; EEG group I) and those with severely abnormal background (n=22; group II). No patient with seizures had a normal EEG background. Monitoring was started after 24 h PP in five neonates in EEG group I (26, 38, 39, 48 and 64 h PP) and in three in group II (25, 39 and 42 h PP). Follow-up information was available for all survivors except one. This neonate had a mildly abnormal EEG and a normal MRI. Another neonate with mild EEG abnormality and small punctuate periventricular hemorrhages on MRI, who died of necrotising enterocolitis and renal failure, was excluded from outcome analysis. The number of patients in both EEG groups who had restricted 10-20 system of electrode placement did not differ significantly (4/20 vs 4/22, $p = 1.0$), nor did the time of start and total duration of the monitoring.

Table 1. Clinical and EEG monitoring characteristics of the neonates (n= 42)

Characteristic	**EEG background gp I (n=20)**	**EEG background gp II (n=22)**	***p* value**
Gestational age: wk	39.4 (36-41)	39.6 (36-42)	0.42
Birth weight: g	3325 (2140-3900)	3350 (2200-4400)	0.36
Females, no. (%)	12 (60)	8 (40)	0.22
Total duration monitoring, h	43.8 (23-96.5)	40.5 (23-80)	0.63
Start Monitoring, h PP	15.8 (1-64)	15.5 (5-42)	0.42
Start seizures, h PP	22.3 (2-68)	18.3 (7-43)	0.32
Patients with sz at start, no. (%)	11 (55)	10 (46)	0.76
End seizures, h PP	39.8 (4-88)	42 (21-118)	0.72
Patients with sz at end, no. (%)	4 (20)	11 (50)	0.06†
Period PP seizures expressed, h	17.5 (1-42)	20.5 (4-76)	0.14
Lidocaine treatment patients no. (%)	4 (20)	13 (59)	0.01†

All numbers are median (range), unless otherwise specified. ***EEG background gp I***: mild to moderately severe abnormality (grades 1-5), ***gp II***: very severe abnormality (grades 6-8). ***wk***: weeks, ***g***: grams, ***h***: hours, ***PP***: post partum. ***Sz at start/end***: patients expressing seizures within 2 h of start/end of monitoring. †Fisher's exact test

Background activity and seizure characteristics (n=42)

The median seizure number per EEG background category is shown in Table 2 a. There was a significant positive correlation between EEG background abnormality on the one hand and total seizure number (Spearman's *rho* 0.73, $p<0.001$) or total seizure burden (*rho* 0.41, $p<0.01$) on the other. There was a significant negative correlation between EEG background abnormality and individual seizure duration (*rho* −0.51, $p<0.01$) and seizure amplitude (*rho* −0.44, $p=0.004$) (also Fig. 3A-C).

Table 2 b summarizes the seizure characteristics for EEG groups I and II separately and, therefore, addresses the association between HIE severity and seizure activity. Significantly more patients in EEG group II continued to express seizures close to the termination of EEG monitoring and also had seizures refractory to midazolam, requiring treatment with lidocaine (Table 1). Total seizure number and seizure burden were significantly increased in patients with severely abnormal EEG background. Contralateral seizure spread and fast and arrhythmic seizures were seen in significantly more patients in this group than in group I. The number of patients expressing >3 seizure foci in both groups was not significantly different.

Table 2

a. Total seizure (sz) number per EEG background grade

EEG background grade (n)	**1** (3)	**2** (9)	**3** (1)	**4** (2)	**5** (5)	**6** (2)	**7** (2)	**8** (18)	Total (42)
Total seizure no. (range)	9 (3-46)	24 (8-109)	168 (-)	38 (26-50)	66 (7-164)	61 (50-72)	155 (129-181)	200 (12-538)	

b. Comparison of sz characteristics in patients without and with very severe EEG background

Characteristic	**EEG background gp I** (n=20)	**EEG background gp II** (n=22)	***p* value**
Total no. of sz	30 (3-168)	194 (12-538)	<0.001*
Max. no. of sz in 24 h	27 (3-168)	177 (12-408)	<0.001*
Total sz burden, min	44.6 (5.1-370.9)	109.3 (17.5-468.7)	0.03*
Max. sz burden in 24 h	44.6(5.1-370.9)	92 (17.5-468.7)	0.06*
Sz duration, sec	97.5 (21-520)	40.5 (20-106)	<0.01*
Longest sz, sec	305 (37-2400)	178 (25-8756)	0.14*
Sz amplitude, µV	80 (30-400)	42.5 (10-150)	0.008*
Sz frequency, Hz	1.5 (1-5)	2 (0.5-8)	0.07 *
Patients with sz spread contralat, n (%)	12 (60)	20 (91)	0.03†
Patients with fast sz (>3Hz), n (%)	1 (5)	11 (50)	0.002†
Patients with >3 sz foci, n (%)	7 (35)	14 (64)	0.12†
Patients with >50% of sz arrhythmic, n, (%)	1 (5)	7 (32)	0.047†
Patients with TBG injury on MRI, n (%)	3 (15)	16 (84)	<0.001†
Patients with poor outcome, n (%)	6 (33)	22 (100)	<0.001†

All numbers are median (range) unless otherwise specified. ***Sz duration***: duration of individual seizures. ***EEG background gp I***: mild to moderately severe abnormality (grades 1-5), ***gp II***: very severe (grades 6-8). ***Max***: maximum. ***Contralat***: to contralateral hemisphere. ***min***: minutes, ***sec***: seconds, * (Mann-Whitney U test). ***TBG***: Thalami-Basal ganglia injury, ***Poor outcome***: Mental Developmental Index < 70, severe cerebral palsy (GMFCS 3-5), or death. †Fisher's exact test. See ***Appendix*** for definition of EEG grades.

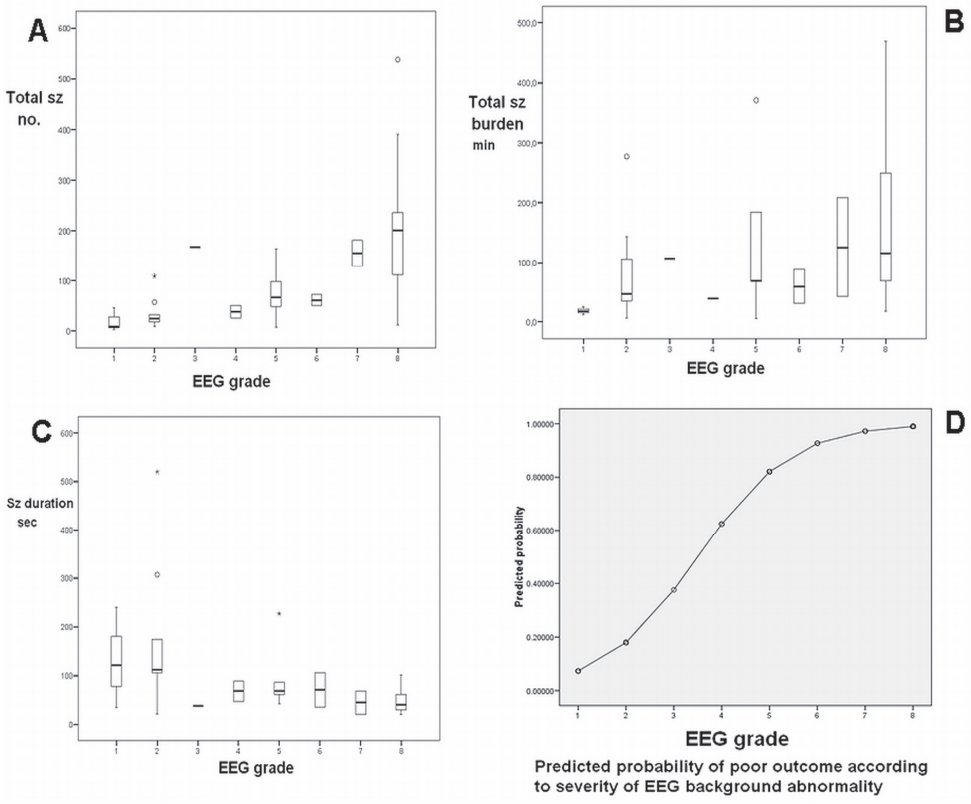


Fig 3. Box plots (A to C) showing the effect of worsening EEG background grade on **A**. total seizure number, **B**. total seizure burden in minutes, and **C**. mean seizure duration in seconds.
D. predicted probability of severely abnormal outcome according to severity of EEG background abnormality.

Outcome (n=40)

There was a very strong correlation between the EEG background grades and outcome categories (Spearman's *rho* 0.79, $p<0.001$). Table 3 provides the number of patients with particular outcome in EEG background grade. Eighteen neonates had the most severe (grade 8) EEG background abnormalities. Of these, seven had isoelectric EEGs and 11 had a persistently low-voltage EEG with long discontinuous periods of more than 20 seconds. All 22 patients with severely abnormal background activity (EEG group II) died. Of these, 17 died after elective withdrawal of ventilator support within 7 days after birth as the prognosis was judged to be extremely poor in them, based on results of multiple parameters including neurological status, EEG, MRI and in some cases, somatosensory evoked potentials.

Table 3. no. of patients with particular outcome in each EEG background grade **(n=42)**

EEG background grade /Outcome category	**1**	**2**	**3**[†]	**4**	**5**	**6**	**7**	**8**	***Total***
1	2	3	-	1	-	-	-	-	6
2	-	3	-	-	-	-	-	-	3
3	-	1	1	-	1	-	-	-	3
4	-	1	-	-	1	-	-	-	2
5	1[*]	-	-	1	3	2	2	18	27

[*]one patient with EEG grade 1, who died due to necrotizing enterocolitis and renal failure was excluded from outcome analysis. [†]Follow-up information was not available for another neonate with EEG grade 3 and a normal MRI. See ***Appendix*** for definition of the 8 EEG grades and text for definition of the 5 outcome categories.

The total seizure number per outcome category is shown in Table 4 a. There was a moderate correlation between outcome on the one hand and the total number of seizures per patient (*rho* 0.61, $p<0.001$) or the seizure burden (cumulative seizure duration, *rho* 0.39, $p=0.01$) on the other. There was also a negative correlation of outcome with seizure amplitude (*rho* −0.37, $p=0.016$) and a trend for a negative correlation with individual seizure duration (*rho* −0.28, $p=0.08$). Comparing the two outcome subgroups (categories 1-3 vs 4-5) showed significantly increased total seizure number and seizure burden in the poor outcome group (Table 4 b). Among the seizure characteristics, only contralateral spread of seizures was significantly increased in patients with poor outcome, while there was a trend for more patients in this group to express fast and arrhythmic seizures.

When the dichotomous grouping of patients according to severity of background activity was done somewhat differently (group I= EEG grades 1 to 4, n=15 and group II= EEG grades 5 to 8, n= 27), the results obtained for the various seizure characteristics were comparable, in relation to both background activity and outcome (data not shown).

Logistic regression analysis with poor outcome as the dependent variable showed a significant effect of the grade of EEG background abnormality (Wald 8.69, $p=0.003$, Exp(B) = 2.64). An important finding in the context of this study was that there was no additional significant effect for (*log*)total seizure number (Wald 0.09, $p=0.76$, Exp(B) = 1.2) or for (*log*)total seizure burden (Wald 2.43, $p=0.12$, Exp(B)= 0.38). That is, severely abnormal outcome can be predicted on the basis of background EEG abnormalities alone. The probability of poor outcome per EEG grade is shown in Fig 3D.

To be able to add MRI abnormalities to this model, exact binary logistic regression with poor outcome as the dependent variable was done using LogXact statistical program version 4.0.2 (Cytel Software Corporation, Cambridge, MA, USA) for Windows™. For this purpose, EEG background activity was used as a continuous variable and presence of basal ganglia and thalamus injury as a categorical covariate. This showed no additional significant effect for the presence of the MRI abnormality ($p=0.182$).

Table 4

a. Median seizure (sz) no. per outcome category

Outcome category (n)	**1** (6)	**2** (3)	**3** (3)	**4** (2)	**5** (27)	Total (40)
Total seizure no. (range)	35 (3-109)	18 (13-28)	57 (7-168)	49 (32-66)	164 (9-538)	

b. Comparison of sz characteristics in patients without and with severely abnormal outcome.

Characteristic	*Mild to mod (n=12)*	*Severe (n=28)*	*p value*
Total no. of sz	26 (3-168)	160 (12-538)	<0.001*
Total sz burden, min	40.8 (5.1-143.5)	109.3 (17.5-468.7)	0.009*
Sz duration, sec	76.5 (21-307)	59 (20-520)	0.194
Sz amplitude, µV	90 (30-400)	50 (10-200)	0.03*
Patients with sz spread contralat, n (%)	6 (50)	26 (93)	<0.01†
Patients with fast sz (>3Hz), n (%)	1 (8)	11 (39)	0.07†
Patients with >3 sz foci, n (%)	4 (33)	17 (61)	0.17
Patients with >50% of sz arrhythmic, n, (%)	0 (0)	8 (29)	0.08†
Patients with TBG injury on MRI, n (%)	0 (0)	19 (76)	<0.001†

All numbers are median (range) unless otherwise specified. ***Mild to mod***: Normal or mild to moderate sequelae (mild CP, GMFCS 1-2), ***Severe***: poor outcome (Mental Developmental Index (BSID MDI) < 70 or severe CP (GMFCS 3-5) or death. ***min***: minutes, * (Mann- Whitney U test). ***Contralat***: to contralateral hemisphere. † Fisher's exact test. ***TBG***: Thalami-Basal ganglia injury. See text for the definition of the 5 outcome categories.

Outcome prediction in patients with mild to moderate HIE

Here we excluded 22 patients with the most severe EEG background abnormality (grades 6-8) to better study the effect of electrographic seizures on outcome. Within this group of patients (EEG group I, grades 1 to 5, n=18), total seizure burden was significantly greater in patients with poor outcome than in those with a good outcome (Table 5). There was also a trend towards significance for increased expression of seizures with contralateral spread in patients with poor outcome. Correlation analysis revealed a trend towards significance for total seizure burden (Spearman's *rho* 0.46, p=0.055) to be associated with poor outcome, but not for total seizure number (*rho* 0.36, p=0.14), individual seizure duration (*rho* -0.14, p=0.59) or seizure amplitude (*rho* -0.11, p=0.65). There was a strong effect of EEG background abnormality in this group also, as seen by the positive correlation between EEG background score and outcome categories (*rho* 0.73, p=0.001). This effect was predominantly due to 4 of the 5 patients with grade 5 EEG abnormality who had a very poor outcome (3 died and 1 developed severe handicap). Repeating the correlation analysis after excluding these 5 patients showed a weak trend for association of increased seizure burden with poor outcome, which was statistically not significant (data not shown).

Table 5. Comparison of seizure (sz) characteristics in patients without & with poor outcome and with mild to moderately severe EEG background (grade 1–5, n=18)

Characteristic	*Mild to mod (n=12)*	*Severe (n=6)*	*p value*
Total no. of sz	26 (3-168)	57 (26-164)	0.13
Total sz burden, min	40.8 (5.1-143.5)	126.9 (38.7-370.9)	0.049*
Sz duration, sec	76.5 (21-307)	87.5 (62-520)	0.35
Sz amplitude, µV	90 (30-400)	80 (35-200)	0.58
Patients with sz spread contralat, n (%)	6 (50)	6 (100)	0.054†
Patients with fast sz (>3Hz), n (%)	1 (8)	0 (0)	1.0
Patients with >3 sz foci, n (%)	4 (33)	3 (50)	0.63
Patients with >50% of sz arrhythmic, n (%)	0 (0)	1 (17)	0.33
Patients with TBG injury on MRI, n (%)	0 (0)	3 (50)	0.029†

All values are median (range) unless otherwise specified. ***Mild to mod***: Normal or mild to moderate sequelae (mild cerebral palsy, GMFCS 1-2), ***Severe***: (Mental Developmental Index (BSID MDI) < 70, severe CP (GMFCS 3-5), or death. ***min***: minutes, ***sec***: seconds, * Mann- Whitney U= 15, ***Contralat***: to contralateral hemisphere. ***TBG***: Thalami-Basal ganglia injury, † Fisher's exact test.

DISCUSSION

Our study reveals strong relationships between total seizure number, cumulative seizure duration (total seizure burden), individual seizure duration, and morphological characteristics of seizures with abnormality of the EEG background. These relationships have not been previously reported. Furthermore, the EEG background strongly predicted poor outcome. Neither total seizure number nor MRI confirmation of lesions of basal ganglia and thalami significantly added to this. Our findings imply that studies measuring seizure burden in isolation and relating it to outcome, omit clinically relevant information that is expressed through EEG background activity and electrographic seizure patterns. These parameters should be included in future studies that assess the effect of postasphyxial neonatal seizures or their treatment on outcome.

Association of abnormal background activity with electrographic and clinical seizures has been found in both human [6] and animal [12] studies. It has also been reported that there is an association of post-asphyxial seizure severity with brain injury as detected by histopathology in newborn piglets [12] in excess of 'structural brain damage' as detected by MRI. It is debatable whether this brain injury that is unmeasured by MRI exists prior to onset of electrographic seizures or is (partly) caused by the seizure activity. It can be seen from our data that patients with higher seizure burdens have more severe hypoxic brain injury as supported by the more severe EEG background grades as well as higher frequency of occurrence of injury to basal ganglia and thalami on MRI. Higher basal nuclei MRI score has also been reported in patients with seizures in previous studies[13,14]. Our results are also in agreement with those of Bye et al.[4], who found that in neonates with electrographic seizures, EEG background abnormality was the only significant predictor of survival at one month. However, unlike these authors and for unknown reasons, we did not find an association between the number of seizure foci and outcome.

Significantly more patients with severe EEG background had seizures refractory to second line AED, requiring treatment with lidocaine. Seizures refractory to multiple AEDs have been found to be associated with poor outcome in both population-based[29] as well as hospital-based[30] studies. We add to this that the severity of the underlying brain injury may be a determining factor of AED refractoriness in acute provoked neonatal seizures.

A somewhat surprising finding in the present study was that individual seizure duration decreased with increasing EEG background abnormality. The usual seizure duration in neonatal encephalopathy is reported to be about two minutes [31]. We add to this, that the

duration of individual seizures expressed on a severely abnormal EEG background is significantly shorter (40.5 vs 97.5 seconds). Use of AED as well as various factors at cellular and network level may influence seizure termination [32,33] and we can only speculate about the reasons for this finding.

Relation between seizure characteristics and outcome

The association between spread of seizures to the contralateral hemisphere and poor outcome as observed in our study has been previously reported [16]. We add that this phenomenon appears to be related to the severity of the EEG background abnormality, i.e., the severity of the brain injury. Our results indicate that the same is true for fast (>3 Hz) seizures. These seizures are easy to recognize, as neonatal seizures predominantly express a frequency around 1 Hz. This association has not been previously reported. In an earlier study, we have observed that fast seizures were predominantly found over the temporal regions [34], an observation that may be related to the well-described occurrence of ictal rhythms of 5-7 Hz in mesial temporal epilepsy[35]. This might suggest that various neonatal seizure patterns could be electrographic signatures for the region of brain involved as well as for the degree of underlying brain injury. Thirdly, arrhythmicity of more than 50% of a patient's seizures was also associated with severe background abnormality and showed a trend towards significance for association with poor outcome. Because both severely disturbed cerebral function and treatment with AED like lidocaine may increase ictal arrhythmicity, this finding is somewhat difficult to interpret. However, we observed rhythmic seizures even in patients with a persistently low voltage EEG background, suggesting that the neural substrates that express seizures and physiological EEG background differ. Lastly, the observed association of lower amplitude of seizures with more severe EEG background and poor outcome is readily explained by the generalized suppression of voltage of all EEG phenomena with increasing severity of encephalopathy.

Our findings thus indicate that the morphological as well as spatial and temporal characteristics of neonatal seizures are influenced by the severity of underlying brain injury. However, a previous study of neonatal seizures that included both preterm and term infants did not find a relationship between ictal patterns and underlying pathology [36]. This discrepancy may be explained by the fact that these authors did not carry out a detailed analysis of the regions of the brain involved and the ictal characteristics. We expect that further insight into the pathophysiology of the above mentioned seizure patterns can be gained

by correlating them with the type of brain injury and studying in detail the anatomical regions involved on MRI. This is the topic of a subsequent study.

Applications

All neonates in the present study with persistent severe EEG abnormality (grades 6-8) died. Also, significantly more patients in this group showed lesions involving the basal ganglia and thalami. This confirms previous work which demonstrated that severe background EEG abnormality that does not recover over 48 h predicts poor outcome [8]. Intensive EEG monitoring and treatment of seizures is not expected to have a beneficial effect in such patients. In an increasingly cost-conscious society, labor-intensive and expensive tests such as EEG monitoring have to be used selectively. Furthermore, treating a severely brain-injured neonate for prolonged periods with AED, only based on the fact that electrographic seizure discharges are continuing to be expressed on EEG, is likely to delay the decision making process of whether to continue advanced life support or not.

We believe that the current study clearly demonstrates the value of long-term EEG monitoring for studying neonatal seizures and we highly recommend its use for both patient care and further research. EEG monitoring is labour-intensive as well as expensive and few centers have access to this. Moreover, expertise to interpret the large amounts of EEG data is not available around the clock in most NICUs. Therefore, we consider the development and use of reliable automated background classification methods in conjunction with seizure detection [22] and localisation [37] algorithms the best way forward.

Strengths and limitations

Strengths of our study include more complete sampling of seizures by continuous EEG monitoring and interpreting them in the context of EEG background abnormalities. We also show the relevance of studying various seizure characteristics in detail, which may have a bearing on the outcome. It is important to recognize, however, that our results are applicable for predicting severely abnormal outcome. They do not address long-term outcome aspects such as school performance, development of epilepsy, behavioural problems etc., which may be more strongly related to neonatal seizures. For this purpose, longer follow-up of survivors is essential.

It could be argued that higher seizure burden as well as more intensive treatment with AED like lidocaine could have adversely affected the EEG background and delayed its recovery in our patients. However, the majority of our patients with severely abnormal background activity had this before the onset of seizures and their treatment. There was also no significant difference between the two EEG groups in the number of patients showing seizures close to

the start of monitoring (Table 1). We recognize that lack of uniformity in the start and end of EEG monitoring could have resulted in underestimation of the seizure burden. This reflects the reality in a tertiary hospital, where many of the neonates are out-born or EEG monitoring is delayed until hemodynamic stabilization is attained. However, we consider it unlikely that this variability in timing has significantly influenced our finding, because the majority of the neonates who continued to have seizures until the end of monitoring were in the severely abnormal EEG group.

Our eight grade EEG background classification has not been validated in a previous study. However, the very strong correlation that we found between the EEG grades and outcome categories suggests that this grading system is a reasonable one. In the patients whom we monitored in 2003, the Sarnat scores [2] were not recorded and this prevented us from comparing the encephalopathy scores of patients with their EEG grades. Selection bias is also a potential confounder. Relatively more patients with very severe encephalopathy were included in this study, and hence the results cannot be generalized to all patients with neonatal HIE and seizures. Furthermore, 17/22 patients in group II died in the first week PP after elective withdrawal of treatment. This may have resulted in a self-fulfilling prophecy, as EEG was one of the parameters used to make this decision. However, such decisions were always taken based on multiple parameters, including neurological status, EEG background, and brain MRI in all cases and somatosensory evoked potentials in some.

Can we use EEG monitoring to identify neonates who may benefit from aggressive treatment with AED?

This question is difficult to answer, as long as our understanding of the pathophysiology of post-asphyxial neonatal seizures remains incomplete. The fact that the effects of aggressive treatment with AEDs on the developing brain are incompletely understood[38] only adds to the complexity of the decision making process. For choosing any treatment, it needs to be shown that the benefits significantly outweigh the risks. Quantifying various seizure parameters and assessing their relationship to outcome is especially interesting in neonates with mild to moderately abnormal EEG background (grades 1-5). These patients are expected to have better outcomes when compared to patients with more severe encephalopathy. It is thus important to study in them whether seizures cause additional brain injury in the context of HIE, as has been shown in studies in animals[39,40]. Although our study was able to demonstrate several trends relating seizure characteristics and outcome, a prospective randomized controlled study is needed to assess the effect of these characteristics and impact of AED treatment on outcome in this patient subgroup.

In conclusion, our results suggest that in future studies of treatment and outcome of persistent neonatal electrographic seizures, stratification of patients according to severity of underlying encephalopathy, based on EEG background abnormalities is indicated.

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2.2 EEG characteristics of postasphyxial neonatal seizures relate to location and pattern of brain injury on MRI

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ABSTRACT

Aim: To describe in detail the spatial and morphological characteristics of electrographic seizures in neonatal hypoxic ischemic encephalopathy (HIE) and to correlate them with cerebral lesions seen on MRI.

Methods: EEG background and electrographic characteristics of postasphyxial seizures were studied in 43 term neonates using video-EEG polygraphy, recorded for ≥24 hours, starting <24 hours post partum. MRI brain was done in 39 and the findings were compared with 36 neonates with HIE, but without seizures recorded during EEG monitoring. Survivors were followed for at least two years.

Results: Majority of the seizures showed central (59.3%) and/or temporal (49.7%) involvement, independent of severity of brain injury on MRI. Frontal seizures tended to be more frequent in patients with injury to thalami and basal ganglia (TBG). Rhythmic sinusoidal delta oscillations were seen only over the central and occipital regions. Rhythmic fast (>3Hz) seizures were seen exclusively over the temporal regions. 75% of seizure patients with asymmetric brain lesions had more seizures lateralized to the side showing more extensive injury. Comparing seizure and non-seizure groups, there was no significant difference in the number of patients showing either TBG, extensive cortical, isolated white matter or isolated cortical injury patterns. However, significantly more patients in the non-seizure group showed relative sparing of the cerebral cortex.

Conclusions: Our findings suggest an effect of anatomical location on seizure morphology. There was a trend for TBG injury to be associated with frontal seizures. Majority of patients with post-asphyxial seizures show cerebral cortical injury on MRI. The occurrence of seizures in patients with relative sparing of cerebral cortex as well as the variable expression of seizures in patients with similar appearing brain injury patterns suggest the limitation of presently used MRI techniques in delineating hypoxic brain injury.

INTRODUCTION

Seizures are a frequent and sometimes the only manifestation of brain dysfunction in the neonate. There is a strong association of neonatal seizures with death or permanent handicap in the survivors[1]. Hypoxic ischemic encephalopathy (HIE) is the most common cause of seizures[2] occurring in the neonatal ICU (NICU). The two best suited investigations to assess the severity of brain injury as well as the prognosis in HIE are EEG[3] and MRI[4]. EEG is best done within 24 hours of birth and its predictive value decreases with time[5]. Continuous EEG monitoring (cEEG) gives two important types of (interdependent) information: changes in background activity reflecting the severity and evolution of the brain dysfunction and the presence of seizures, and is valuable in guiding treatment. It is well known that there is increased chance of occurrence of seizures when the EEG background is abnormal[6]. However, why some neonates with similar severity of HIE develop seizures while the others do not is not well-understood.

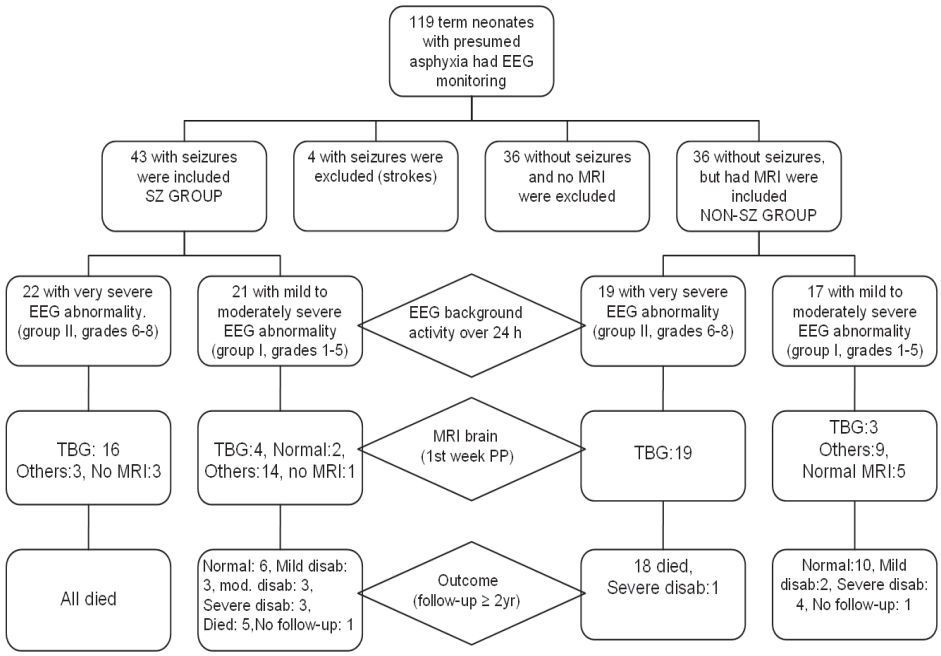
Even though previous studies have described in detail the spatial and temporal characteristics of neonatal seizures due to various etiologies[7,8], correlation with neuroimaging was lacking. Also, no study has systematically compared the electrographic characteristics of postasphyxial neonatal seizures recorded by cEEG with the abnormalities seen on MRI. Aim of this study was to describe in detail the spatial and morphological characteristics of post-asphyxial neonatal seizures recorded by cEEG and to determine if there was any relation between the EEG characteristics and the pattern of brain injury seen on MRI. Secondary aim was to ascertain differences, if any, between MRI patterns of brain injury seen in neonatal HIE, in term newborns with and without seizures. With this, we aim to improve the understanding of the pathogenesis of seizures induced by neonatal hypoxic brain injury.

METHODS

EEG data from 119 consecutive term newborns with presumed perinatal asphyxia, who underwent video-EEG monitoring for ≥24 hours (h) in the NICU, between March 2003 and August 2007 as part of an ongoing study, were reviewed. The inclusion criteria for EEG monitoring were: gestational age of 37-43 weeks with clinical features of encephalopathy, and having at least one of the following features of birth asphyxia[9]: a) arterial pH of umbilical cord blood ≤7.1, b) Apgar score ≤5 at 5 minutes, and c) high clinical suspicion (like fetal distress, umbilical cord prolapse, difficult labour, or a history of convulsions). The inclusion criteria for presumed asphyxia were kept broad so as not to miss patients with postasphyxial

seizures. Babies with congenital cardiac abnormalities and multiple congenital anomalies, were excluded. None were treated with hypothermia. Electrographic seizures were present in 47. Four neonates with ischemic stroke involving well-defined arterial territories on MRI, were excluded. Data from the remaining 43 (seizure group) were used for analysis. They were compared with 36 neonates who underwent EEG monitoring, but had no recorded seizures and subsequently had an MRI of the brain (non-seizure group). Fig 1 shows the scheme of inclusion of patients. The study had the approval of the Erasmus MC Medical Ethical Review Board.

Fig 1. Inclusion and follow-up of 43 neonates with (sz group) and 36 without (non-sz group) electrographic seizures. *h*: hours, *PP*: post partum, *TBG*: Thalami-Basal Ganglia injury, *yr*: years, *disab*: disability.



EEG registrations

Digital video-EEG with polygraphy, started mostly ≤24 h post partum (PP), was registered continuously for 1-3 days using a Nervus^{TM} monitor (Taugagreining hf, Reykjavik, Iceland). Scalp electrodes were applied according to the 10-20 International System[10]. In eight patients, we used a restricted 10-20 system using 13 electrodes (Fig 4). EEG sampling frequency was 256 Hz. Band-pass filter was 0.3 -70 Hz.

EEGs were interpreted by a clinical neurophysiologist as part of patient care and this information was used to make treatment decisions.

Scoring EEG background: We used an eight grade system (***Appendix***), derived from literature[11-14] and modified based on our clinical experience. This scoring system emphasizes discontinuity (voltage and length of the discontinuous periods) and its evolution over 24 hours, presence of variability, reactivity and sleep-wake cycling (SWC). Severity of discontinuity is fairly easy to assess and has been shown to be a robust parameter to predict outcome[15]. Discontinuity was defined as voltage attenuation (< 25μV for mild and <10 μV for severe) in at least 50% of the EEG channels, lasting for ≥5 seconds. To score discontinuity, the most frequently occurring measure of the amplitude and duration of the discontinuous periods were used. We omitted parameters such as frequency content and duration of the EEG bursts, frequency of sporadic or rhythmic sharp waves within and outside the bursts, because we wanted the classification to be readily applicable at the bedside. EEGs were reviewed and background activity scored by a clinical neurophysiologist (PJC) together with a neonatologist (RMS). We also sub-classified patients into two groups based on EEG background abnormalities [EEG group I: normal to moderately severe (grades 0-5) and EEG group II: severely abnormal (grades 6-8)], for the sake of statistical analysis. All the data were digitally available for review. Consensus was reached in all cases.

Electrographic seizures: were defined as a clear variation from background activity, displaying a repetitive pattern of oscillations or sharp waves or a mixture of both, lasting ≥10 seconds, with evolution in amplitude and frequency over time [8,16,17]. Inter-rater agreement (PJC and GHV) among two experienced clinical neurophysiologists for scoring of seizure occurrence in one hour segments of EEGs from 10 patients was 73% [17], with a *Kappa* value of 0.4. Subsequently, this data was reviewed jointly by both and the scored seizures were agreed upon by consensus.

For this study, the EEGs were reviewed in their entirety by a neurologist/clinical neurophysiologist (PJC), blinded to the clinical and MRI results, and electrographic seizures were scored for their onset, location, spread, frequency and rhythmicity. Seizures were considered focal when they remained confined to one EEG electrode and regional when they involved two or more adjacent electrodes. We defined other ictal parameters as below.

Contralateral spread: An electrographic seizure that develops over contralateral homologous areas during or within 5 seconds of termination of a seizure discharge, expressing similar morphology and frequency, but evolving independently[18] .

Fast seizure: Electrographic discharge expressing a dominant frequency of >3Hz (these seizures had a slower onset of ≤2 Hz), lasting ≥10 seconds (Fig 5). This pattern differs from the majority of neonatal seizures that have a slower frequency. Seizures that showed a short burst of fast activity (5–14Hz) lasting for 3 to 5 seconds, followed by a slower dominant frequency of 0.5-2 Hz were not included.

Arrhythmic seizure: marked variability in the interval between individual complexes in a seizure discharge for the major part of its duration.

Neuroimaging

All newborns had serial cranial ultrasound scans during their stay in the NICU. MRI scans of the brain were done in the first week PP in 39 patients with seizures and in 36 without seizures, according to an asphyxia protocol on a 1.5 Tesla scanner. All scan sessions included conventional T1 & T2-weighted spin-echo, proton density images, as well as diffusion weighted images (DWI) with apparent diffusion coefficient (ADC) mapping. Images were scored using information from all sequences, by an experienced pediatric neuroradiologist (ML) who was unaware of the results of the EEGs and outcomes, according to six brain injury patterns (***Appendix***)[19]. Pattern 7 included injury patterns not fitting one of these six prototypes. We also noted if there was presence of extensive cortical injury, defined as bilateral extensive lesions involving at least three cortical regions. Predominant involvement of the rolandic cortex as well as relative sparing of cerebral cortex, when present, were also noted. Based on these MRI patterns, patients were further clustered into two classes, depending on whether there was injury to deep grey matter (thalamus and striatum, TBG), which is a good predictor of neurological outcome[4].

Outcome

A pediatrician unaware of the EEG results performed follow-up examinations until at least two years of age. Bayley Scales of Infant Development (BSID)[20] were used. Those in whom cerebral palsy (CP) was detected were referred to the pediatric neurologist. Patients with a normal short-term development at one year, who were not further followed at our clinic, were assessed with a telephone questionnaire based on the Pediatric Stroke Outcome Measure[21] administered to the parents. The Gross Motor Functional Classification System (GMFCS)[22] was used to classify the severity of CP. Outcome was classified into five categories. 1= normal, 2= mild motor disability (no CP) or language/cognitive delay, 3= moderate disability, CP (GMFCS 1 or 2), 4=severe disability (GMFCS 3-5, Mental Developmental Index [BSID MDI] < 70) and 5= dead. For the purpose of statistical analysis, these five groups were further sub-classified into two, as good/mildly abnormal (categories 1-3) and poor (categories 4-5) outcomes.

Statistical analysis

Association between two or more groups was studied using Spearman's rank correlation. Dichotomous classification of patients based on EEG background abnormalities [EEG group I: normal to moderately severe (grades 0-5) and EEG group II: severely abnormal (grades 6-8)] as well as based on outcome [normal to moderate abnormality (categories 0 to 3) and poor (categories 4 & 5)] was done for statistical analysis. Presence of various MRI abnormalities was also compared between the seizure and non-seizure groups. Comparison of two groups was done using Mann-Whitney U or Fisher's exact (2-tailed) tests. All tests were performed using SPSS^{TM} version 15.0. A p value of <0.05 was considered significant.

RESULTS

The median (range) gestational age of the newborns was 40 (36-42) weeks and the birth weight was 3325 (2140-4400) grams. EEG monitoring was started 15.5 (1-64) hours after birth and continued for 42 (23-96) hours. The EEG and MRI findings as well as the outcome of the patients are given in Tables 1 to 4. Monitoring was started within 24h in the majority of patients. However, it was done after 24 h PP in five neonates in EEG group I (26, 38, 39, 48 and 64h PP) and in three in group II (25, 39 and 42h PP). Follow-up information was available for all survivors except one. This neonate had a mildly abnormal EEG and a normal MRI. The number of patients with seizures in both EEG groups who had restricted 10-20

system of electrode placement did not differ significantly (4/21 vs 4/22, p = 1.0), nor did the time of start and total duration of the monitoring. There were no significant differences between the seizure and the non-seizure groups with respect to gender, birth weight, Apgar scores, time of start and duration of EEG monitoring. EEG monitoring data of 1864 h was reviewed. Eighteen neonates in the seizure group and 17 in the non-seizure group had the most severe (grade 8) EEG background abnormalities. Of these, 12 had isoelectric EEGs and 23 had a persistently low-voltage EEG with long discontinuous periods of more than 20 seconds. MRI scans were performed at a median of 4 days (range 2 -14) after birth. The position of EEG electrodes with respect to brain regions was ascertained in one patient (Fig 2) and this corresponded to what has been described previously in adults[23].

Table 1. EEG characteristics of seizures (sz), MRI patterns of brain injury and outcome in patients with mild to moderately abnormal EEG background (grades 1 to 5)

no.	EEG	morph	dur	loc	spread	freq	amp	MRI pattern	outcome
1	1	shw	240	C-3	T-L	1	150	**5**: F-L>R,TPO-bil	1
2	1	mixed	121	C, O2	none	1	200	**7**: pv, Rol	5
3	1	mixed	34	C-bil, F-L	T-bil	2	70	**7**: hge gyrus rectus-L	1
4	2	mixed	174	C-3 > T-bil, O2	PTO-bil	2	100	**0**: edema PT-L>R	2
5	2	shw	40	T-bil, O2	C, contra T	5	50	**0**: IVH,EDH PO-R	1
6	2	shw	21	Cz-3-4	P3-4	1	50	**4**: bil F,O	2
7	2	shw	112	C4, O2	PT-R	2	100	**4**: FP-R>L, Rol	3
8	2	pshw	520	C-bil	P-bil, T	2	80	**5**: FCPO hge	4
9	2	shw	106	CT-bil,PTO-R	F-L	2	100	**5**: bil F,T,P,O	1
10	2	pshw	307	T-C-L>R	contra T-C	2	80	**5**: F-bil, T-L>R	2
11	2	mixed	108	Cz-C3	PT-L	2	60	No MRI	NA
12	2	osc	127	C4,TO-bil	T-bil	1	400	**7**: PO-L>R	1
13	3	osc	38	C,T-bil	O-bil	1	200	**1**: F-L>R, insula	3
14	4	shw	18	O2, FT-L,	PT	1	65	**1**: Rol	4
15	4	mixed	89	O1	O2	2	40	**3**: FP-bil,SDH TO-L	5
16	4	shw	47	C-bil, PTO-bil	TP-bil	2	30	**6**: bil-PT>FO	1
17	5	shw	227	Cz, C-bil, T-L	P-bil, T-R	2	50	**6**: bil-FPO,T-L	5
18	5	shw	86	TPO-L>R	contra TPO	1	35	**2**: FO-L>R	5
19	5	shw	62	Cz-4,T4,TO-bil	contra TO	1	100	**1**: O-bil	4
20	5	osc	68	C-bil,O-bil	F-bil, T	2	100	**6**: bil Rol,O, insula	5
21	5	shw	43	C4, F7-T3	T4, C3	2	50	**5**: TO,insula-R>L	3

EEG: background grades (1-8), morph: predominant Sz morphology, *osc*: sinusoidal oscillations, *shw*: sharp waves, *mixed*: mixture of both osc and shw, *pshw*: positive sharp waves. *Dur*: mean duration of individual sz (sec), *loc*:EEG electrode location of sz, *C*: central, *F*:frontal, *P*:parietal *T*: temporal, *O*: occipital, *L*:left, *R*: right, *bil*: bilateral, *spread*:sz spread, *contra*: contralateral hemisphere, *freq*: frequency (Hz), *Amp*: amplitude(μV), *MRI pattern*: (nos. in bold) scored according to 6 injury patterns, *7*: other patterns not included in the 6 main categories, *IVH*: intraverntricular hemorrhage, *EDH*: extradural hemorrhage, *pv*: periventricular, *Rol*: rolandic, *SDH*: subdural hemorrhage, *hge*: hemorrhagic lesions. *NA*: not available. *Outcome categories (1-5)*: see text.

Table 2. EEG characteristics of seizures (sz), MRI patterns of brain injury and outcome in patients with severely abnormal EEG background (grades 6 to 8)

no.	EEG	morf	dur	loc	spread	freq	amp	MRI pattern	outcome
1	6	mixed	35	C,T-bil	F-bil,TO	3	35	**1**: Rol,brainstem, vermis	5
2	6	shw	106	Cz,C3-4,T-bil	contra, F3-4	2	25	**2**: F-bil, TO-L>R	5
3	7	mixed	20	FC-bil, FT-bil	contra	7	15	**1**: cx spared	5
4	7	mixed	69	CPT,F-bil,O-bil	contra	1	150	**2**: F,insula,amygHc-bil,Rol	5
5	8	mixed	37	Cz	C3,4,T-L	1	150	**2**: bil-F,T,insula,Rol,O	5
6	8	mixed	20	PTO-L>R, FT-bil	contra	8	10	**2**: brainstem, F-bil	5
7	8	osc	20	F-bil	C-bil	4	30	**1**: F-bil, Rol	5
8	8	osc	32	F- bil, C-bil	contra F	1	150	**2**: ext.cx, WC	5
9	8	shw	33	FT-L>R	contraT	5	20	no MRI	5
10	8	mixed	30	C3,4, F7,8, O2	contra CT	3	25	**2**: ext.cx, WC, brainstem	5
11	8	mixed	25	Cz-3-4, F-bil	contra FC,T	6	25	no MRI	5
12	8	mixed	75	FT-bil, Fp2-F4	contraT	3	30	**2**: ext cx, WC	5
13	8	mixed	64	Cz, T-bil	contraT, F-L	1	50	**2**: ext.cx, WC	5
14	8	mixed	25	O-R>L,T5,T6	contra, C3	2	50	**3**: Rol, P-R>L	5
15	8	mixed	62	C3,T-bil,O-bil	none	2	70	**2**: F-L>R,T-bil,insula,Rol	5
16	8	mixed	40	C3-4-z,T-bil,O2	contra CT	3	75	**1**: Rol-L>R	5
17	8	mixed	60	T,C,F-bil	contra	2	50	**2**: ext. cx, max FT-bil	5
18	8	osc	102	C-L,T-R	none	1	50	**2**: ext.cx, WC	5
19	8	pshw	41	Cz,T3,4, O1,2	contra T,O	1	30	**2**: ext.cx, WC	5
20	8	osc	50	C-bil, F-bil	T-bil,contra	3	70	**1**: FP-bil, Rol	5
21	8	mixed	58	PTO-L>R, FT-R>L	contra	2	20	no MRI	5
22	8	mixed	77	F-bil,T-L	contra F, C3	1	150	**2**: ext.cx, WC,(Rol spared)	5

EEG: background grades (1-8), morph: predominant sz morphology, *osc*: sinusoidal oscillations, *shw*: sharp waves, *mixed*: mixture of both osc and shw, *pshw*: positive sharp waves. *Dur*: duration (sec), *loc*:EEG electrode location of sz, *C*: central, *F*:frontal, *P*:parietal *T*: temporal, *O*: occipital, *L*:left, *R*: right, *bil*: bilateral, *spread*: sz spread, *contra*: contralateral hemisphere, *freq*: frequency (Hz), *Amp*: amplitude(μV), *MRI pattern*: (nos. in bold) scored according to 6 injury patterns, *7*: other patterns not included in the 6 main categories, *Rol*: rolandic, ext.cx: extensive cortical injury, WC: 'white cerebrum' seen on DWI , amygHc: amygdala-hippocampus. *Outcome categories (1-5)*: see text

Table 3. EEG background grades, MRI findings and outcomes in asphyxiated neonates without seizures and mild to moderate abnormality of EEG background (grades 1-5)

no.	EEG	MRI pattern	outcome
1	1	**0**: normal MRI	1
2	1	**0**: mild injury globus pallidus, fimbriae fx -bil	1
3	1	**0**: mild WM edema	1
4	1	**0**: mild WS injury P-L	1
5	1	**0:** mild WS injury P-bil, F-R>L	1
6	1	**4**: WS FPO, mild PTO	4
7	1	**4**: WS FP-bil, WM (parasagittal)	1
8	1	**4**: WS & cx F-bil,PO-R>L	1
9	1	**4**: WS F-R>L, PO-bil	1
10	2	**5**: WM mild hges, pv L>R, Rol hgic infarct, mild SDH PTO-R	1
11	2	**7**: mild WS O-bil, small pv bleeds	NA
12	2	**7**: mild iv, pv hge, small SDH infratentorial	2
13	3	**1**: cx relatively spared	4
14	3	**4**: mild WS F P O L>R, small SDH infratentorial	1
15	4	**1**: cx relatively spared	4
16	4	**1**: cx relatively spared, cerebral peduncles	4
17	4	**4**: WS FTP-bil	2

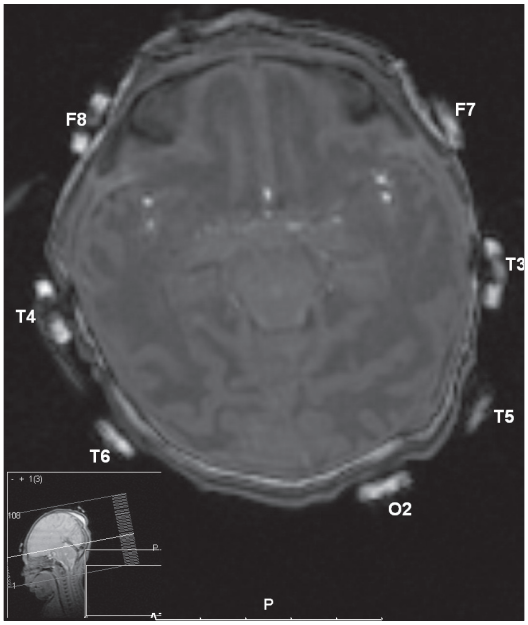
EEG: background grades (1-8), *F*:frontal, *P*:parietal *T*: temporal, *O*: occipital, *L*:left, *R*: right, *bil*: bilateral, *MRI pattern*: (nos. in bold) scored according to 6 injury patterns, *7*: other patterns not included in the 6 main categories, *WM*: white matter, *fx*: fornix, *WS*: watershed, *Rol*: rolandic, *ext.cx*: extensive cortical injury, *WC*: 'white cerebrum' seen on DWI , *cx. spared*: relative sparing of cerebral cortex, *hge*: hemorrhage, *hgic*: hemorrhagic, *iv*: intraventricular, *pv*: periventricular, *SDH*: subdural hemorrhage, *NA*: not available. *Outcome categories (1-5)*: see text.

Table 4. EEG background grades, MRI findings and outcomes in asphyxiated neonates without seizures and severe abnormality of EEG background (grades 6-8)

no.	EEG	MRI pattern	Outcome
1	7	**2**: WC	5
2	7	**2**: brainstem, cx relatively spared	5
3	8	**2**: WC	5
4	8	**2**: ext.cx.	5
5	8	**1**: mild Rol,mild F-L>R	5
6	8	**1**: mesial T-bil, mild Rol, brainstem	5
7	8	**2**: ext. cx., max. FP>O	5
8	8	**2**: WC	5
9	8	**2**: ext. cx., F>P>O, WM, cerebellum	5
10	8	**2**: Rol, Hc,T-bil, O, brainstem	5
11	8	**2**: mild Rol, F-R, small cerebellar hge, brainstem, WM	5
12	8	**2**: ext.cx, FP-R>L	5
13	8	**2**: ext.cx.	5
14	8	**2**: ext. WS injury	5
15	8	**1**: Rol-bil(severe)	5
16	8	**2**: Rol, insula-bil	5
17	8	**2**: WC	5
18	8	**2**: WC, brainstem	5
19	8	**2**: cx relatively spared	4

EEG: background grades(1-8), *F*:frontal, *P*:parietal *T*: temporal, *O*: occipital, *L*:left, *R*: right, *bil*: bilateral, *MRI pattern*: (nos. in bold) scored according to 6 injury patterns, *7*: other patterns not included in the 6 main categories, *Rol*: rolandic, *ext.cx*: extensive cortical injury, *WM*: white matter, *Hc*: hippocampus, *WS*: watershed, *WC*:'white cerebrum' seen on DWI , *cx. spared*: relative sparing of cerebral cortex, *hge*: hemorrhage, *Outcome categories (1-5)*: see text.

Fig 2. Gradient echo MR image in axial plane showing the position of MRI compatible EEG electrodes (Patient no. 16, Table 4) in relation to inferior frontal (F7,8), temporal (T3,4, 5&6) and occipital (O2) brain regions. The anatomical level of the section is indicated at the bottom left.

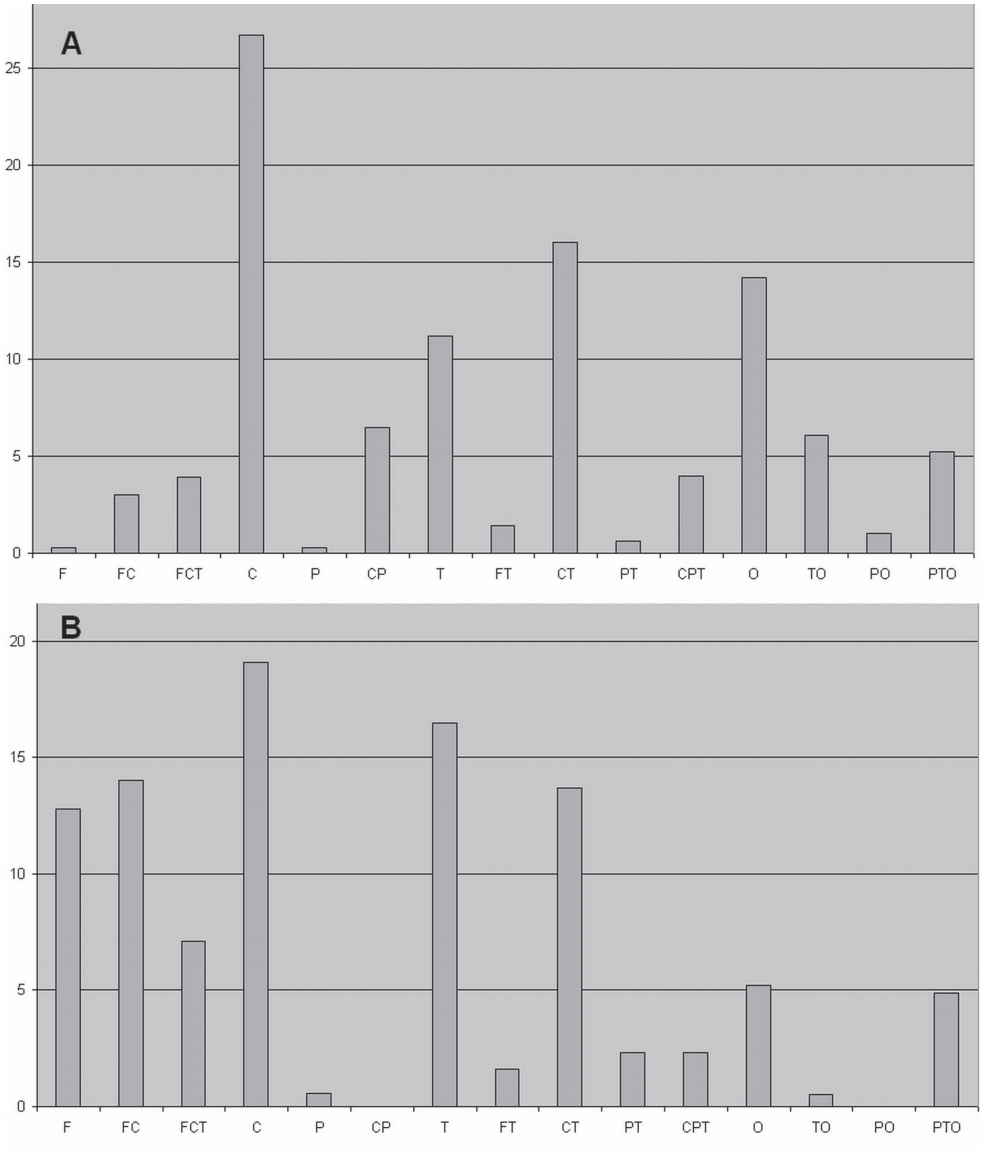


Seizure parameters

A total of 5061 seizures were recorded (median 72 per patient, range 3-538). Seizures started 19 (2-68) h PP and continued for 18.8 (1-76) h. The median cumulative duration of all seizures (seizure burden) was 70 (2.1-469) minutes. The median values (range) for various seizure parameters were: firing frequency 1.5 (1-8) Hz, individual seizure duration 60 (18-520) sec, and amplitude 50 (10-400) μV.

Location of seizures: The localisation of seizure foci in patients in EEG groups I and II are given in tables 1 and 2 and the percentages of seizures per brain region are shown in Fig 3. The single most common seizure focus (seizure remaining focal without spreading) was central, seen in 23.3% of seizures, followed by temporal (14.2%). When regional seizures were also considered, central region involvement was seen in 59.3% of seizures while temporal regions were involved in 49.7%. Purely focal seizures occurring over the occipital (9.8%) and frontal (6.9%) regions were less common, and only one patient each showed all seizures confined to either frontal or occipital regions. Comparing patients in the two EEG groups (I, mild to moderate background abnormality vs II, severely abnormal background), there was no difference in the central and temporal region preponderance of seizures (Fig 3A and B). However, in patients with very severely abnormal EEG background (group II) when compared with group I, there was a trend for more patients to show more than 50% of seizures localised to the frontal regions, (6/22 vs 1/21, p=0.095).

Fig 3. Distribution of seizures per EEG electrode or per region, expressed as percentage, in patients with (**A**) mild to moderate (grades 1-5, n=21) and (**B**) severe (grades 6-8, n=22) EEG background abnormality. Central and temporal regions showed seizures most frequently in both groups. There was a trend for more patients in **B** to express seizures over the frontal regions (p=0.095).



Slow (1 to 2 Hz) rhythmic oscillatory seizures: This pattern was seen over the central regions (Fig 4) in five patients and occipital regions in two. No other region exhibited this pattern.

Fast seizures: Ten patients expressed fast (>3Hz) seizures. Majority (9/10) of these patients had severe abnormalities of EEG background (EEG group II). The seizure activity was rhythmic in three patients while in six, it was arrhythmic. The rhythmic fast seizures were seen exclusively over the temporal regions (Fig 5) while arrhythmic fast seizures were expressed variably, over frontal, central, temporal or occipital regions.

Fig 4. **A**. Rhythmic oscillatory slow (1.5Hz) seizure over the central region (patient no. 5, Table 2). The vertical grey bar over the aEEG trends corresponds to the EEG page displayed. EEG background is severely abnormal (grade 8). Seizures (112 recorded) started 11h post partum and continued for 9h (duration of monitoring 24h). MRI pattern 2: T1weighted spin-echo images (**B, C**) show hyperintense signal changes in the frontal region, basal ganglia and thalami. DWI (**D**) and FLAIR (**E**) images show extensive cortical and subcortical injury bilaterally. Patient died on the 7th day.

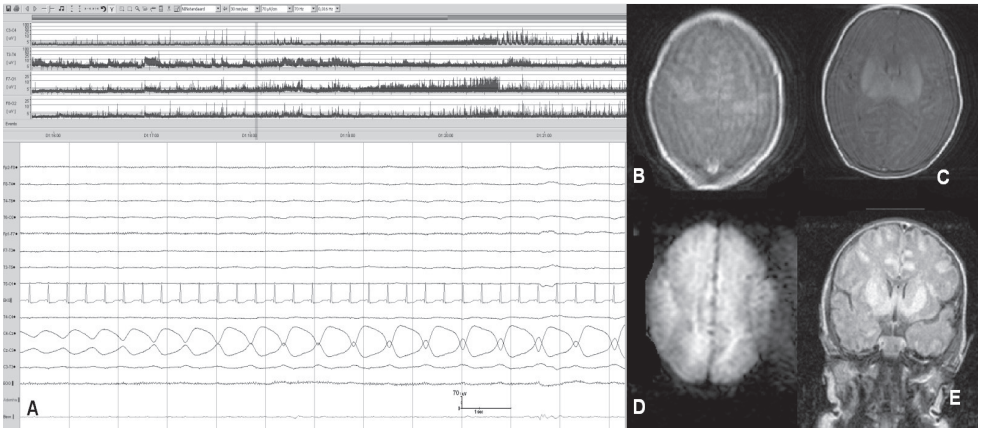
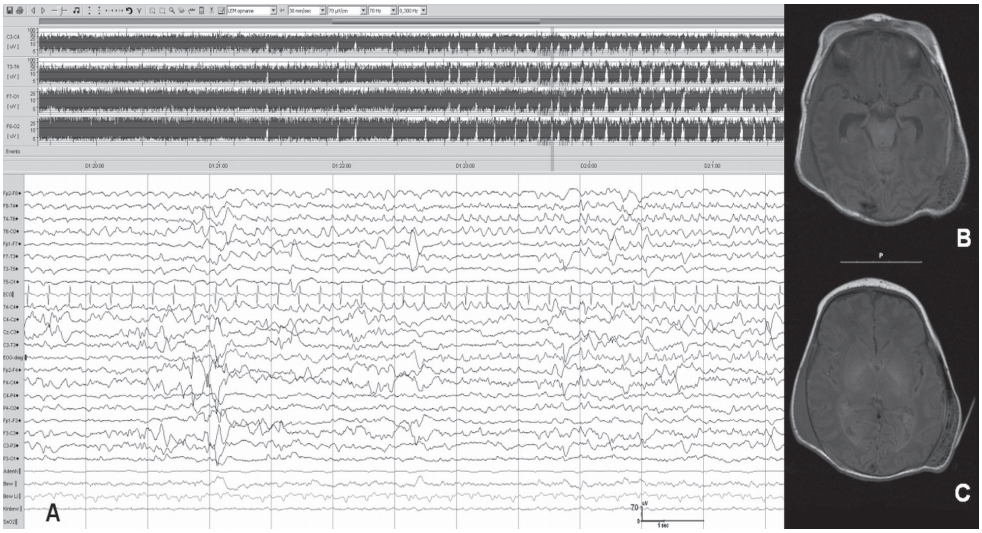


Fig 5. **A**: Rhythmic fast (5-6 Hz) seizure over right temporal region (patient no. 5, Table 1). The vertical grey bar over the aEEG trends corresponds to the EEG page displayed. EEG background was moderately abnormal (grade 2). Seizures (109 recorded) started 26 h post partum and continued for 18 h (duration of monitoring 96 h). **B & C**: MRI shows a mild left temporal subdural hemorrhage, a small extradural hematoma over right parieto-temporal region as well as small intraventricular hemorrhages, but no obvious parenchymal injury. Patient was normal at 2 year follow-up.



Comparison of MRI patterns of brain injury in seizure and non-seizure patients

Comparison of MRI findings between the seizure and non-seizure groups is shown in Table 5.

Injury to deep grey matter is associated with severe EEG background abnormality: When seizure and non-seizure groups were compared, there was no significant difference in the number of patients showing TBG injury. In both groups, significantly more patients with severe abnormality of EEG background showed injury to TBG (seizure patients: EEG group II vs I, 19/19 vs 5/20, p=<0.001, non-seizure patients: EEG group II vs I, 19/19 vs 3/17, p= <0.001).

Presence of extensive cortical injury: When seizure and non-seizure groups were compared, there was no significant difference in the number of patients showing extensive cortical injury. However, in both groups, an association of this MRI pattern with TBG injury was seen. (extensive cortical injury in seizure patients with TBG injury vs those without, 10/24 vs 2/15, p=0.08; extensive cortical injury in non-seizure patients with TBG injury vs those without, 11/22 vs 0/14, p= 0.002). (Fig 4)

Relative sparing of the cerebral cortex: Relative sparing of the cerebral cortex was seen in significantly more patients without seizures; 10/36 newborns in the non-seizure group (5 had normal MRIs and 5 had TBG injury with minimal cortical injury) vs 3/39 patients with seizures (2 with normal MRIs and 1 with TBG injury and minimal cortical injury), p=0.03.

Presence of asymmetric brain injury: There was a trend (p=0.09) for more patients in the seizure group (when compared to the non-seizure group) to show this pattern of injury. Of the 16 patients in the seizure group showing this MRI pattern, 12 (75%) showed preponderance of seizures over the hemisphere with more extensive injury on MRI.

Predominant injury to white matter (pattern 5): There was no significant difference between the seizure and non-seizure groups (5/39 vs 1/36) in the number of patients showing this injury pattern. All the patients with this relatively milder pattern of brain injury had also less severe EEG background abnormality (all belonged to EEG group I).

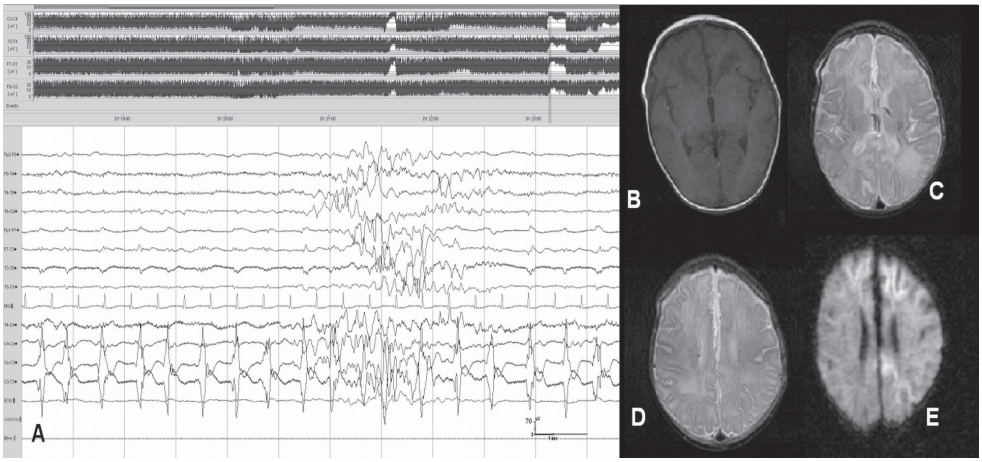
Seizure pattern in 4 of the 5 patients with this injury consisted of slow (1 to 1.5Hz), rhythmic, high amplitude (150-250μV) activity, consisting predominantly of positive sharp waves in the central regions (Fig 6). However, 3 patients with extensive injury to basal ganglia- thalami and cortex (Pattern 2) as well as 2 patients with watershed injuries (Pattern 4) also showed similar seizure patterns.

Table 5. Comparison of patterns of brain injury seen on MRI in patients with (Sz group) and without (Non-sz group) electrographic seizures.

MRI Pattern	*Sz group* n=39	*Non-sz group* n=36	*p value*
Basal ganglia-Thalami injury	24 (62)	22 (61)	1.0
Extensive cortical injury	12 (31)	12 (33)	1.0
Relative sparing of cortex	3 (8)	10 (28)	0.03*
Asymmetric brain injury	16 (41)	8 (22)	0.09
Predominant WM injury	5 (13)	1 (3)	0.20
Isolated cortical injury	3 (8)	0 (0)	0.24
Isolated watershed injury	2 (5)	6 (17)	0.14
Predominant rolandic injury†	11 (28)	9 (25)	0.80

Percentages are given in parentheses. WM: white matter. *Fisher's exact test. †Patients with injury to rolandic cortex, after excluding those with extensive cortical injury.

Fig 6. **A.** Slow (1.5Hz) rhythmic seizure with predominantly positive sharp wave morphology over the central region (patient no. 10, Table 1). The vertical grey bar over the aEEG trends corresponds to the EEG page displayed. EEG background is moderately abnormal (grade 2). Seizures (28 recorded) started 40h PP and continued for 24h. MRI pattern 5, predominant white matter injury, seen best on T1weighted (**B**) and T2 weighted (**C, D**) spin-echo images. DWI (**E**) shows white matter and watershed injury. Patient has mild disability (outcome category 2: language delay and behavioural problems) at 2 year follow-up.



Isolated cortical injury: This pattern was seen exclusively in the seizure group, in three patients with moderate abnormalities of the EEG background.

Isolated watershed injury: Even though more patients in the non-seizure group showed this pattern when compared to the seizure group, the difference was not statistically significant.

All patients with this relatively milder pattern of injury had only mild to moderate EEG background abnormalities (EEG group I).

Rolandic cortex injury: There was no significant difference in the number of patients in the seizure and non-seizure groups showing injury to rolandic cortex (after excluding patients with extensive cortical injury).

Outcome related to EEG characteristics and MRI patterns

TBG injury is related to poor outcome: In patients with this severe brain injury pattern, outcome was not related to the additional presence of seizures. In the non-seizure group, 22/23 patients with poor outcome had lesions of TBG, while in the seizure group, this figure was 24/30 (not significantly different).

Isolated white matter injury and outcome: Two of the five patients in the seizure group (nos. 1 & 9, Table 1) and the one patient in the non-seizure group developed normally. Poor outcome was seen in only one patient with this pattern of brain injury. This patient (no. 8, Table 1) in the seizure group had bilateral extensive multifocal symmetric white matter hemorrhages with patent venous sinuses and deep veins. A high (277 minutes) seizure burden was recorded by cEEG during the NICU stay, and at one year of age, patient had developed severe mental retardation and refractory epilepsy.

Isolated cortical injury and outcome: Two of these patients (nos.17, 20, Table 1) had high seizure burdens (185 & 371 minutes) and more extensive cortical injury on MRI, and both died in the neonatal period. The third neonate (no. 16, Table 1) had a more restricted pattern of brain injury involving predominantly the parieto-temporal regions and a lower seizure burden (39 minutes). This patient was normal at 2-year follow-up.

Relatively spared cerebral cortex and outcome: Among the patients with normal MRI, 5/5 patients in the non-seizure group and 1/2 in the seizure group developed normally. One patient (no. 4, Table 1) in the seizure group had a mild developmental delay. All the patients who had injury to TBG with relative sparing of cerebral cortex in both seizure and non-seizure groups developed severe handicap or died.

DISCUSSION

This study presents one of the largest data sets of neonatal seizures to date, occurring in the context of presumed perinatal HIE, recorded using continuous EEG monitoring and its correlation with MRI patterns of brain injury.

Spatial and morphological characteristics of seizures

Postasphyxial seizures predominantly involve central and temporal regions: Majority of seizures (focal as well as regional) in our patients showed central and temporal involvement. This is similar to what is reported in the literature [8,24-26]. Vulnerability of perirolandic and temporal regions to hypoxic injury and seizures may be due to various reasons. Increased glucose metabolism in these regions as well as thalamus and cingulate cortex have been shown in the neonate using PET studies[27-29]. The parasagittal watershed regions are vulnerable to injury not only after hypotensive episodes, but also in neonatal HIE[30]. Seizures also may lead to local increases in cerebral blood flow and may lead to insufficient flow-metabolism coupling[31,32], further compounding the injury. Vulnerability of peri-rolandic cortex has also been shown in MRI studies of perinatal HIE[33]. Temporal region, especially the hippocampus is well known to be associated with 'epileptogensis'[34].

Frontal region seizures tend to associate with severe brain injury: We noted a trend towards significance of occurrence of more seizures in the frontal regions, associated with TBG injury and more severe EEG background (Fig 3B). Conversely, one might state that in patients with less severe brain injury (EEG background grades 1-5), frontal regions are relatively spared. It is attractive to speculate that the lower cerebral blood flow and glucose utilization rates in the frontal regions in neonates (it increases only by 6 to 12 months of age[27]) make these regions less vulnerable to injury.

Application: The information about predominant locations of seizure foci is relevant in the choice of electrodes when long-term EEG monitoring is planned in perinatal asphyxia, either with multichannel aEEG (Fig. 4, 5 & 6) or conventional EEG using a restricted 10-20 system. It is expected that at least 90% of the seizures will be detected by the central (Cz, 3,4), temporal (T3,4) and occipital (O1,2) electrodes together.

Seizure morphology and location: Two types of seizure patterns are worth noting. Rhythmic slow delta oscillations were only seen over the central (Fig 4) and occipital regions. Patrizi et al. have noted a preponderance of similar appearing seizure patterns in preterm infants, as compared to term infants[26]. We also observed rhythmic fast seizures exclusively over the temporal regions in three neonates. These patterns are reminiscent of the 5 to 7 Hz ictal patterns described in mesial temporal lobe epilepsy[35]. In a previous study, we have observed that such patterns are consistently associated with ictal heart rate changes, suggesting the close structural and functional relationship of this ictal pattern with temporo-limbic structures[36].

MRI patterns of brain injury in patients with seizures

Comparing the seizure and non-seizure groups, we did not find any difference in the occurrence of TBG injury. In contrast, Glass et al.[37] found that patients with 'severe seizures' had predominant injury to the basal nuclei. This discrepancy may be explained by the fact that their patients did not undergo continuous EEG monitoring and thus the estimation of 'seizure severity' may be erroneous. We also found a strong association of extensive cortical injury with TBG injury in both seizure and non-seizure patients. Brain injury patterns vary with the duration and severity of the hypoxic ischemic insult (and also the timing of MRI) and this group with TBG and extensive cortical injury may constitute the extreme end of the spectrum. Many of these patients also expressed extensive abnormalities on DWI sequences, which has been referred to as the 'white cerebrum' in literature[38].

Asymmetric brain injury in seizure patients: The close relationship of lateralized cortical injury and lateralized predominance of seizures in 12/16 patients in our seizure group suggests that cortical injury is closely related to generation of acute provoked seizures in perinatal HIE. Another noteworthy point is that among seizure patients, significantly more newborns in EEG group I (mild to moderate background abnormalities) showed asymmetric brain injury when compared to EEG group II. This can be explained by the strong association of TBG lesions with severe abnormalities of EEG background (EEG group II), which by definition is a symmetrical pattern of brain injury. Extending this line of thinking, one can understand the occurrence of more symmetrical cortical lesions in this group of patients.

Isolated white matter injury (Leukomalacia): This has been described in literature as one of the patterns of term HIE[39,40]. Pathogenesis of these lesions in term newborns may be different from the periventricular leukomalacia seen in preterm infants[41]. We observed a peculiar ictal pattern consisting of high amplitude rhythmic positive sharp waves at 1-1.5 Hz, predominantly involving the central regions in 4 of the 5 patients with this injury pattern. However, as this ictal pattern was also seen in few patients with other type of brain injuries, we are unable to conclude if this is an electrographic marker for injury to white matter and watershed regions. Pooling of data from other centers will help to answer this question.

MRI findings in non-seizure patients:
Not surprisingly, relative sparing of the cerebral cortex was seen in significantly more patients without seizures, when compared to the seizure group. There was also a tendency for more patients in the non-seizure group to show watershed lesions, as has been previously reported[37].

Limitations of the study: EEG monitoring was started after 24 hours in some of the patients and we may have missed seizures in them. More patients with seizures tended to have MRI studies and this could have lead to selection bias. MRI was not done in 36 neonates who underwent EEG monitoring but had no seizures recorded. Some of the non-seizure patients without MRI died in the early neonatal period while some made a rapid clinical recovery from their encephalopathy, and this could have resulted in mainly survivors of moderate and severe encephalopathy undergoing imaging in the non-seizure group. However, we think that this is less likely, as the proportion of patients showing TBG or extensive cortical injury in both groups was comparable. The MRI studies were cross-sectional in nature and were not performed sequentially. Hence we are not able to comment on the timing of brain injury in many of our patients.

The combination of EEG background activity evolution and MRI abnormalities has been shown to provide an accurate diagnosis of the site and severity of brain injury and prognosis[42-44]. We add to this that studying the electrographic seizure characteristics using EEG monitoring gives useful additional information. Our findings also suggest that ictal morphology may be related to both the anatomical location as well as the severity of brain injury. There are gaps in the current knowledge about the pathologic substrates of post-asphyxial neonatal seizures. Our findings, similar to previous studies[45,46], suggest that they

tend to occur more in the context of cortical injury visible on MRI. However, the occurrence of relative sparing of the cerebral cortex on MRI in 8% of patients with seizures also shows the limitation of MRI in detecting some types of brain injury. It is also not clear why patients with similar appearing brain injury on MRI variably express seizures. Brain injury 'unseen' by conventional MRI techniques or other patient characteristics like genetic susceptibility may play a role in this. Newer MR techniques like perfusion imaging may show differences in reperfusion (hyperperfusion) that may correlate with seizure location and may help to improve outcome prediction in such patients[47].

The diagnostic significance of co-localised electrographic abnormalities and pattern of brain injury on MRI, especially in paradigms like isolated leukomalacia and isolated cortical injury, need to be studied in a larger cohort prospectively, with longer follow-up to assess the prognosis for cognitive outcome, development of epilepsy etc.

In addition, insight into the brain substrates of neonatal seizures as well as improved outcome predictions may be achieved by automated source localization[48] of the seizures using a realistic head model[49] as well as quantitative studies of brain lesion volume[50,51].

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2.3. Heart rate changes are insensitive for detecting postasphyxial neonatal seizures

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based on

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ABSTRACT

We studied heart rate (HR) changes during 169 seizures (mean 12 per patient, range 8-18) in 14 neonates with severe birth asphyxia. HR changes were found in 21 seizures (12.4%) of 8 patients (HR increases in 4, decreases in 1 and both patterns in 3 patients), suggesting the existence of neonatal cerebral hemispheric connections with brainstem autonomic regulatory centers. HR monitoring appears to be insensitive for detecting post-asphyxial neonatal seizures.

INTRODUCTION

Seizures are a common manifestation of central nervous system (CNS) dysfunction in newborn babies and are associated with poor outcome[1]. Seizures are often subclinical, detectable only by EEG. Depending on their location, seizures may affect heart function. Heart rate (HR) monitoring has therefore been suggested as method for seizure detection[2]. Relative immaturity of the newborn brain may result in cardiac responses to seizures that differ from those seen in older patients, but no study has systematically addressed this issue. We studied HR during seizures in newborn babies that underwent EEG monitoring after birth asphyxia to a) determine whether ictal HR changes occur in them, and b) assess the potential of HR monitoring as a tool to support seizure detection.

METHODS

We reviewed EEG and EKG data from 40 full-term neonates with severe birth asphyxia.

The inclusion criteria for EEG monitoring were:

Newborns with a gestational age of 37-43 weeks and having at least one of the following features of birth asphyxia

a) arterial *pH* of umbilical cord blood $\leq$ 7.1,

b) Apgar score $\leq$5 at 5 minutes, and

c) high clinical suspicion of asphyxia

(like fetal distress, umbilical cord prolapse, difficult labor, with or without history of convulsions).

Babies with congenital cardiac abnormalities and multiple congenital anomalies were excluded from the study. The 15 babies that showed electrographic seizures[3] had received parenteral loading doses of phenobarbitone (20mg/kg) and subsequently midazolam (0.1mg/kg), followed by maintenance doses of midazolam, varying from 0.1 to 0.5 mg/kg/hour. Brain MRI scans were done between the 3rd to 4th postpartum day in all patients. The study had the approval of the institutional medical ethics committee.

Digital video-EEG with polygraphy was registered continuously for 1-2 days using a Nervus^{TM} monitor (Taugagreining hf, Reykjavik, Iceland). EEG electrodes were placed according to the 10-20 international system. Single-channel EKG was recorded from two electrodes placed over the left and right side of the chest. Sample frequency was 256 Hz for both EEG (band-pass filter 0.5-35 Hz) and EKG channels (band-pass filter 1-70Hz).

All EEGs were reviewed in their entirety, and scored for seizure onset and offset, lateralization, localization, spread, and frequency. For ictal HR analysis, we selected only those ictal discharges that lasted at least 20 seconds and showed clear evolution in frequency and amplitude over time. Seizures with clinical manifestations (three in one patient) were excluded to avoid secondary influence of motor activity on HR.

The R tops of the QRS complexes in the EKG were automatically detected and R-R interval plots were generated using MatLab™ software (The MathWorks, Natick, MA). In these plots, each R-R interval was converted to an instantaneous heart rate (beats per minute) and plotted versus time, from 30 seconds before ictal onset to 60 seconds thereafter. R-peak detection by the computer was visually checked to ascertain that artefacts were excluded and that no R peaks were missed. Ictal HR results for each seizure were compared with those obtained from an interictal "baseline" period. These periods were chosen as 60-second epochs, not showing any epileptiform abnormalities, ending at least one minute before ictal onset, and within 5 minutes preceding each seizure. This procedure controlled to some extent for other variables that might influence HR like sleep, medications, and metabolic parameters.

We determined the SD of the HR variability during the baseline epoch and used it to set an upper and lower limit to the "allowed" variability of the ictal HR. For this purpose, we also determined the mean HR from the first 100 instantaneous HR values in the last minute before ictal onset. Next, upper and lower limits were set at this mean HR $\pm$ 4 $\times$ SD. Any HR change that crossed these limits for at least 10 successive heart beats and occurred between 30 seconds before and 60 seconds after the ictal onset was classified as significant ictal HR increase or decrease. All plots were reviewed by three investigators (PJC, JHB and GHV), and the classification was verified and agreed upon by consensus.

For assessing differences between groups, chi-squared tests were used unless otherwise indicated. All tests were performed using the statistical software package SPSS™ version 10.1. A p value of ≤ 0.05 was considered significant.

RESULTS

Ictal HR analysis could not be performed in one patient due to too many artefacts in the recording. A total of 169 seizures were studied in the remaining14/40 patients with seizures (mean, 12 per patient; range, 8-18). Clinical data are shown in Table 1.

Table 1. Clinical data and ictal heart rate (HR) variability

Patient no./sex	Location of sz	Abnormalities on MRI brain	Baseline HR variability	Ictal HR variability /no. of sz	Outcome/ duration follow-up (months)
1/F	C, T	Cort, subcort	No	0/11	Died
2/F	C, TO	Subcort	Yes	0/9	Died
3/F	C, O, FT	Cort, subcort	No	↑ 4, ↓1/15	Died
4/F	P, FT, C	Cort, subcort	No	0/18	Died
5/M	T, C, F	Cort, subcort	No	↑ 5/18	Died
6/F	C, T	None	Yes	↑ 1, ↓1/10	Good/7
7/F	C, T	Cort, subcort	Yes	↑ 1/10	Died
8/F	C, T	Cort, subcort	No	↓ 3/11	Severe sequelae/4
9/F	TPO	Subcort	No	↑1, ↓1/10	Died
10/M	C, T	Subcort	Yes	↑ 2/13	Motor delay/12
11/M	T, O	Subcort	No	0/10	Died
12/F	C, T	Cort, subcort	Yes	0/10	Severe sequelae/24
13/M	C, T	Cort, subcort	No	0/13	Died
14/M	FCT, TO	None	No	↑ 1/11	Good/6

Sz: seizure, F: frontal, T: temporal, C: central, P: parietal, O: occipital, ↑: Ictal HR increase and ↓: decrease. MRI abnormalities: Cort: hyperintense signal involving most commonly the rolandic and insular cortex. Subcort: signal changes involving thalami, basal ganglia or subcortical white matter.

Table 2. Correlation of ictal heart rate (HR) changes with seizure location and laterality.

Temporal Region involvement		a) Stable baseline			b) Variable baseline			Total a)+b)		
		HR change			HR change			HR change		
		↑	↓	No	↑	↓	No	↑	↓	No
Yes	Right	2	0	15	2	0	15	4	0	30
	Left	7	1	31	2	1	21	9	2	52
No	Right	1	0	28	0	1	2	1	1	30
	Left	1	1	28	0	2	8	1	3	36

↑: Ictal HR increase and ↓: decrease, No: No ictal HR changes.

Ictal HR changes were found in 21/169 seizures (12.4%) in 8/14 patients (Tables 1 and 2). Ictal HR increases were seen in four patients, decreases in one and both patterns in three. These changes sometimes preceded ictal onset by 10-30 seconds. Seizures with temporal and extratemporal involvement equally showed HR changes (15/97 vs 6/72, $p = 0.17$), as did right- and left-sided seizures (6/66 right vs 15/103 left, $p = 0.29$). The numbers of right- and left-sided seizures showing an ictal HR increase (5/66 vs 10/103, $p = 0.63$) or decrease (1/66 vs 5/103, $p = 0.25$) were not different, either. In one exceptional patient (no.5, Table 1), ictal HR consistently increased when the seizures involved temporal regions and had a discharge frequency of 5 to 7 Hz, while no HR changes were seen with slower ictal activity (Figs 1 & 2, Table 3).

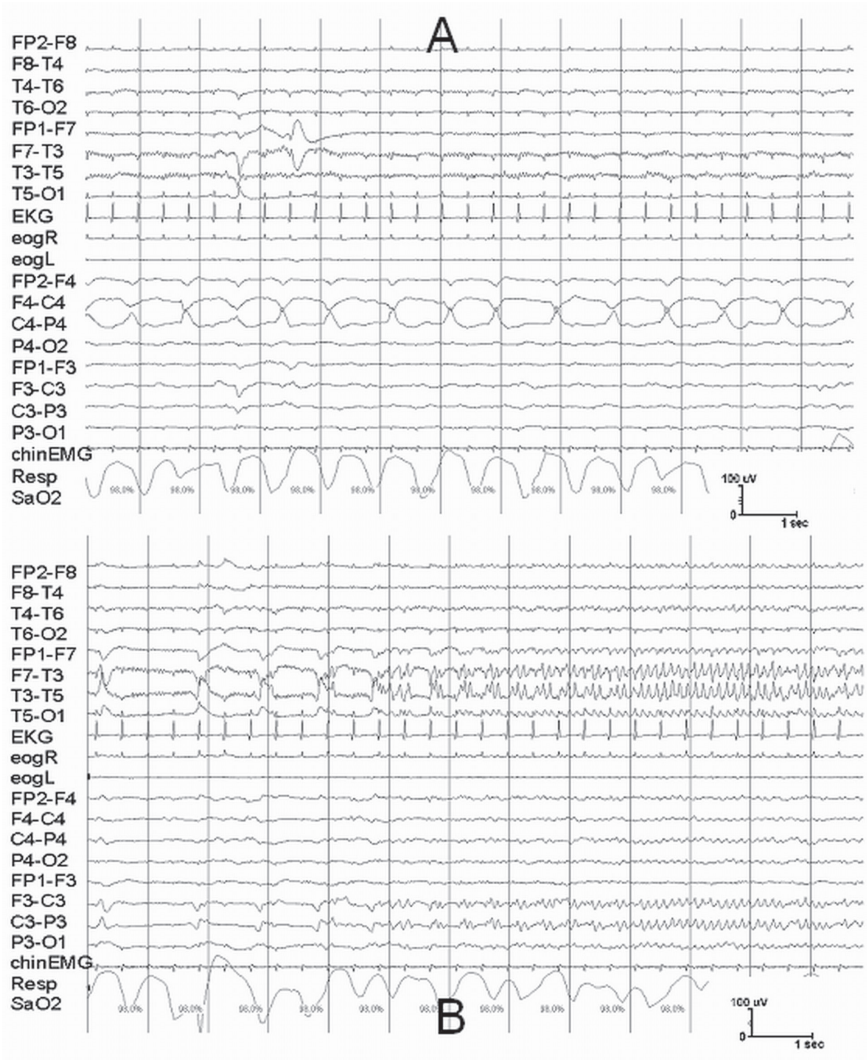

Fig 1. A. Electrographic seizure of 1 Hz frequency, involving the right central region in patient no.5, not associated with ictal HR changes. **B**. Electrographic seizure starting at a frequency of 1 Hz and evolving to reach 6-7 Hz, involving the left temporal region in the same patient. This type of seizure was consistently associated with HR increase.

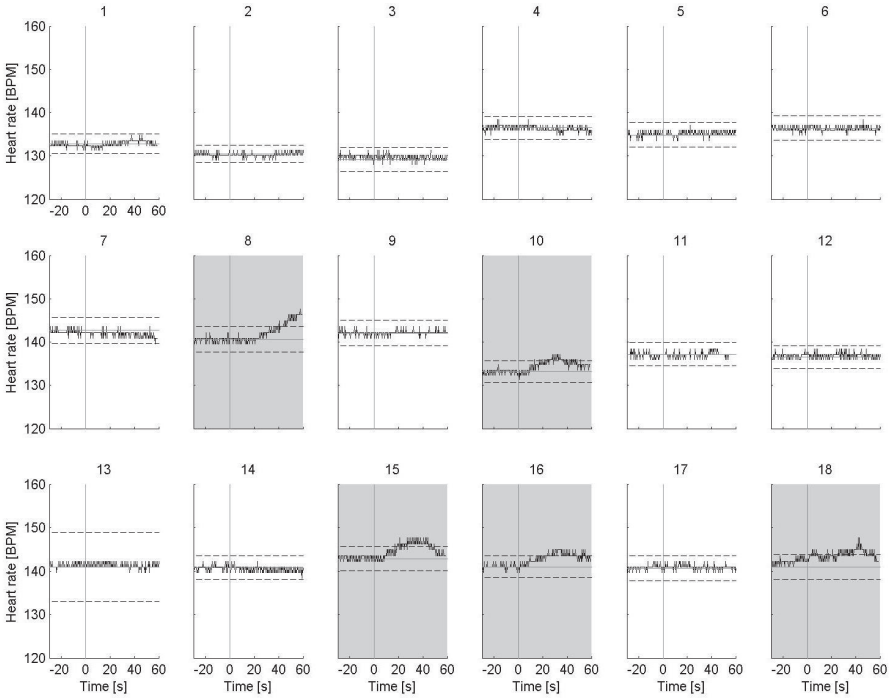

Fig 2. Instantaneous HR (R-R interval) plots of the 18 seizures in patient no.5 described in Tables 2 and 3. Seizures no. 8, 10, 15, 16, and 18 show an ictal HR increase. The vertical line marks the time of onset of the ictal discharges on scalp EEG. The dashed lines above and below the curves denote upper and lower limits (mean + 4SD) of the allowed variability as assessed from the preceding baseline epochs.

Table 3. Details of seizures and ictal heart rate (HR) changes in patient no.5.

Seizure No.	Duration (seconds)	Location		Frequency (Hz) of ictal discharges	Ictal HR changes
		Onset	Spread		
1	45	T, R	T, L	1 to 4	No
2	78	T, R	T, L	1 to 4	No
3	79	C, R	No	1	No
4	70	F, L	No	1	No
5	79	C, R	No	1	No
6	90	F, R	No	1	No
7	55	F, L	No	1	No
8	36	T, L	FC, L	5 to 7	↑
9	164	C, R	No	1	No
10	28	T, L	C, L	5 to 7	↑
11	116	F, R	F, L	1	No
12	165	C, R	No	1	No
13	180	C, R	No	1	No
14	85	C, R	CF, L	1	No
15	34	T, L	C, L	5 to 7	↑
16	34	T, L	C, L	5 to 7	↑
17	200	F, R	No	1	No
18	36	T, L	C, L	5 to 7	↑

F: frontal, T: temporal, C: central, R: right, L: left, ↑: Ictal HR increase

The baseline HR showed two types of patterns: a) preserved beat-to-beat variability, and b) markedly diminished or absent HR variability (stable baseline HR). For this purpose, we defined "stable HR" as a coefficient of variation (SD divided by mean times 100%) of less than 2% in all or all but one of the baseline epochs (Fig 3).

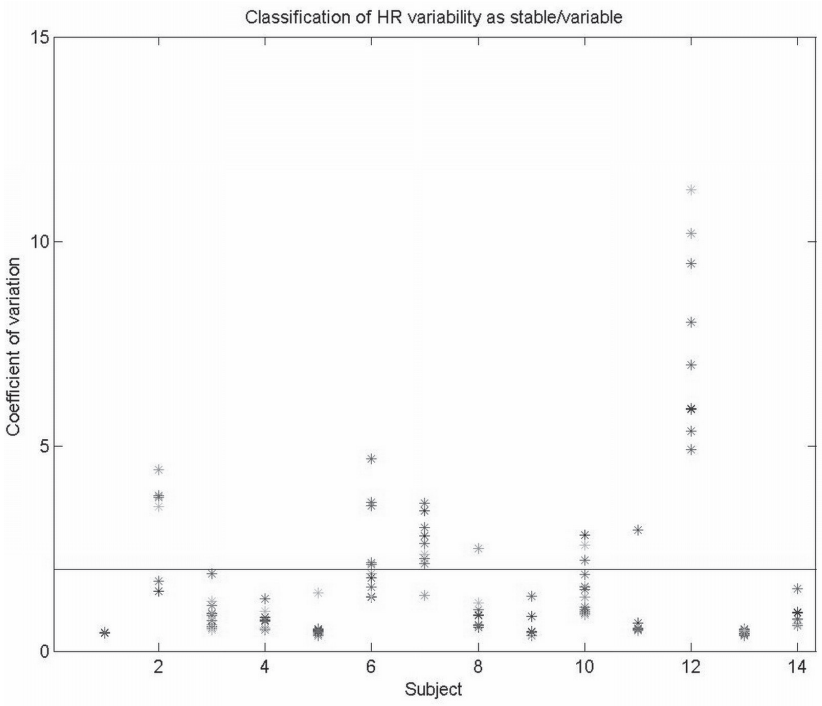

Fig 3. Markedly diminished or absent baseline heart rate variability was seen in patient nos. 1, 3, 4, 5, 8, 9, 11,13 and 14.

There was no difference between the number of patients with a stable or varying baseline HR that showed ictal HR changes (5/9 vs 3/5, p = 0.66, Fisher's exact test). Regarding outcome, there was no difference in mortality between patients with preserved and absent baseline HR variability (2/5 vs 7/9, p = 0.2, Fisher's exact test), nor between patients that did and did not show ictal HR changes (4/8 vs 5/6, p=0.2, Fisher's exact test).

DISCUSSION

We found that ictal HR changes can occur in neonates, providing evidence for the existence of neonatal cerebral hemispheric connections with brainstem autonomic regulatory centers. However, HR monitoring appears to be insensitive for detecting seizures after birth asphyxia. Ictal HR changes were seen in only 12.4% of the seizures, which is much less frequent than reported (73-98%) in older children and adults[2, 4, 5]. This low occurrence may be due to immaturity of the neonatal brain. Alternatively, it may be secondary to hypoxic brain damage, hypoxia-induced abnormal autonomic cardiovascular control, or the use of sedative medications. Antiepileptic drugs and sedative medications are known to suppress autonomic cardiac responses[6], and they have probably influenced our results significantly. These medications differed between patients and could not be controlled for, other than by our use of a baseline epoch.

In our patients as a group, there was no significant effect of seizure lateralization or localization on the occurrence and nature of ictal HR changes. However, one patient who had temporal region seizures consistently showed ictal tachycardia when the ictal discharges had a frequency of 5-7 Hz. This combination of findings might be explained by spread of the temporal seizure discharges to mesio-limbic structures or insula[7], resulting in a higher frequency of ictal discharges[8] and tachycardia.

Diminished HR variability is considered a poor prognostic sign in encephalopathic neonates[9, 10]. However, we found no significant increase in mortality in the stable baseline HR group, probably due to the small numbers of patients in our study. Our finding of ictal HR changes occurring in similar number of patients with preserved and absent baseline HR variability suggests that the CNS substrates responsible for ictal HR changes are relatively insensitive to hypoxia-induced damage.

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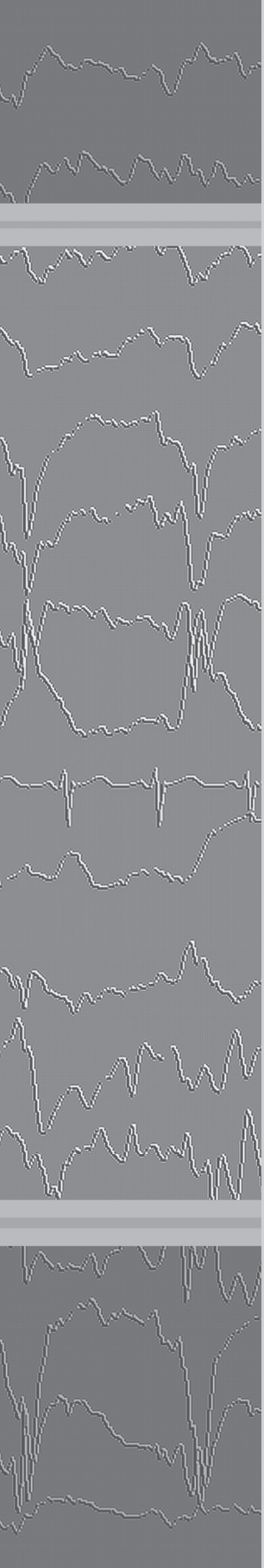

Chapter 3

Development and validation of automated neonatal seizure detection

3.1 Automated neonatal seizure detection mimicking a human observer reading EEG

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ABSTRACT

Objective

The description and evaluation of a novel patient-independent seizure detection for the EEG of the newborn term infant.

Methods

We identified the characteristics of the neonatal seizure by which a human observer is able to detect them. Neonatal seizures were divided into 2 types. For each type, a fully automated detection algorithm was developed based on the identified human observer characteristics. The first algorithm analyzes the correlation between high-energetic segments of the EEG. The second detects increases in low frequency activity (< 8Hz) with high autocorrelation.

Results

The complete algorithm was tested on multi-channel EEG recordings of 21 patients with and 5 patients without electrographic seizures, totaling 217h of EEG. Sensitivity of the combined algorithms was found to be 88%, Positive Predictive Value (*PPV*) 75% and the false positive rate 0.66 per hour.

Conclusions

Our approach to separate neonatal seizures into two types yields a high sensitivity combined with a good *PPV* and much lower false positive rate than previously published algorithms.

Significance

The proposed algorithm significantly improves neonatal seizure detection and monitoring.

INTRODUCTION

Seizures occur in 1 to 3.5/1000 births and are a common sign of neurological disorder in neonates[1]. The causes of seizures are various, with 90% of all cases being attributed to biochemical imbalances within the CNS, hypoxic ischemic encephalopathy, intracranial hemorrhages and infarcts, intracranial infection, and developmental (structural) anomalies. The newborn brain is very susceptible to seizures as term infants have well-developed excitatory mechanisms and poorly developed inhibitory mechanisms[2]. This explains the greater incidence of seizures in the neonatal period than at any other time in life[3]. There is controversy about whether or not seizures cause brain damage. However, an increasing number of studies have shown that neonatal seizures cause lasting changes in the CNS, is related to poor outcome[2,4,5] and late behavioral effects[6,7]. In a strategy of neuroprotection of the newborn, effective detection of these seizures is needed in order to assess their potential additional damage and establish timely treatment. The detection of seizures is usually based on clinical signs in conjunction with visual assessment of the EEG. In neonates, the clinical seizures are often subtle and may be missed without constant supervision[8]. Furthermore, many seizures tend to be subclinical, implying that they can be detected only by EEG monitoring. Combined with the fact that EEG analysis requires particular skills which are not always present around the clock in the Neonatal Intensive Care Unit (NICU) this means that many seizures are missed[2]. Therefore, an automated system that reliably detects neonatal seizures would be of significant value in the NICU.

In the literature, many seizure detection algorithms have been described. The best known methods are based on computing a running autocorrelation function[9], rhythmic discharges detection[10], modeling and complexity analysis[11], and wave-sequence analysis[12]. Others are based on the extraction of features using entropy, wavelets, frequency content, etc., and then training a classifier[13-15] on these features to correctly categorize the EEG. We believe that the classifier approach is not suitable for patient-independent seizure detection. Neonatal seizures have an extremely variable morphology, frequency, and topography, even within the same patient[16,17]. Due to this large variability, training a classifier with fixed properties on a set of features is a too rigid strategy.

Our approach to the detection of neonatal seizures is to develop an algorithm which mimics a human observer reading EEG, as it is essentially the human observer we are trying to replace. We identified two major characteristics of neonatal seizures which lead to their detection by a human observer. The first characteristic is that all seizures represent a clear change relative to

the background EEG. The second and most important characteristic is the repetitiveness of the signal, because nearly all seizures display some kind of recurrent pattern. We aimed to develop an algorithm based on these two characteristics using time domain signal processing, as this is the most straightforward way to mimic the human observer. The implementation is based on several concepts already introduced in the literature, but we combined and improved these and added some of our own concepts.

METHODS

EEG data set

All data were recorded at the Sophia Children's Hospital (part of the University Medical Center Rotterdam, the Netherlands). The dataset consisted of long-term video-EEG recordings of 26 full-term neonates with perinatal asphyxia of which 21 contained electrographic seizures and 5 were seizure-free. Fourteen had the full 10-20 set of electrodes (17 electrodes: Fp1,2, F3,4, C3,4, Cz, P3,4, F7,8, T3,4, T5,6, O1,2), while 12 had a restricted 10-20 set of electrodes (13 electrodes: the above set without F3,4 and P3,4). Each measurement started within 24 hours of birth. Monitoring was done for 24 to 48 hours. Segments of EEG containing seizures and at least eight hours long were selected for each patient. Sampling frequency was 256 Hz. Inter-rater agreement between two experienced clinical neurophysiologists in scoring the number of seizures in one-hour segments of EEGs from 10 patients was 73%. Subsequently, the data was reviewed jointly by both and the scored seizures were agreed upon by consensus. The 8-hour segments of EEGs were then scored for seizure activity by one of the raters. Seizures were scored when they showed clear variation from the background EEG activity lasting for at least 10 s and showed evolution in time and or change in amplitude and frequency. The data set consisted of EEGs with a wide variety of background activity, ranging from low voltage, to burst suppression and normal background activity. No selection has been made regarding artefacts present in the EEG. Before analysis, the data was filtered between 0.3 and 30Hz.

Detection Method

When analyzing the neonatal seizures, we identified two major seizure types. The first type is what we call the spike train seizure type (Fig. 1A); the second is what we call the oscillatory

seizure type (Fig. 1B). Nearly all neonatal seizures can be classified uniquely into one of these types or as a combination of the two types (Fig. 1C). In our dataset, roughly 50% of all seizures consisted of a combination of the two types, 35% were purely consisting of spikes and the remaining 15% were pure oscillatory seizures.

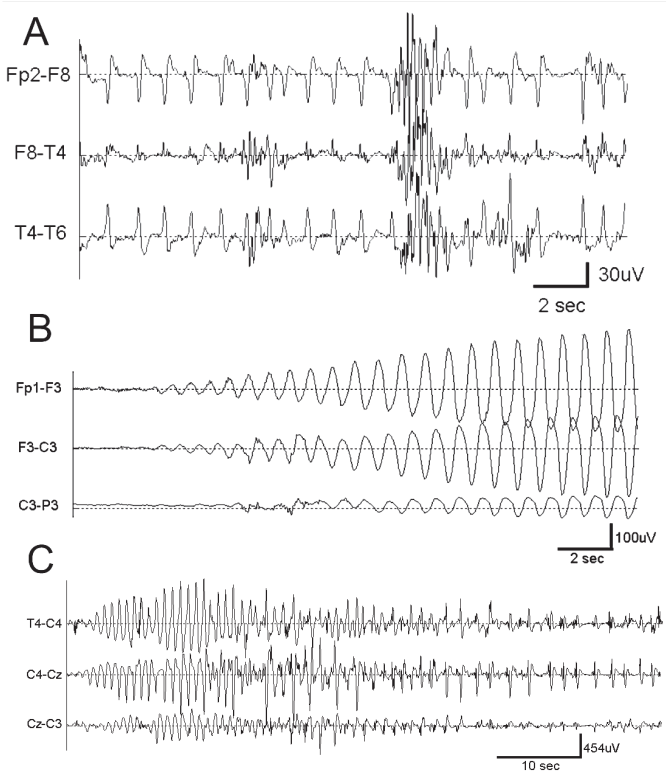

Fig 1A: Example of a spike train type seizure

Fig 1B: Example of an oscillatory type seizure

Fig 1C: Example of a seizure consisting of both morphologies. The seizure starts with oscillatory activity and ends with a spike train.

The major difference between the two types is that the oscillatory type is a fluent, continuous seizure, whereas the spike train type consists of isolated spikes appearing on a background of lower voltage EEG. This means that the oscillatory type has a continuous kind of repetitiveness while the spike train type has a discontinuous kind. Another difference is that the oscillatory activity generally has lower frequency content than the high-frequency spikes.

We need to stress that this categorization into two seizure types is not based on any physiological interpretation, but was adopted purely from a signal processing point of view. A detection algorithm was developed for each seizure type separately. During analysis, both algorithms run in parallel and detection occurs if one or both of the algorithms detects a

seizure. The two algorithms are calculated on each EEG-channel separately. When detection occurs in one channel, the complete EEG at that time instance is regarded as seizure activity, independent of the detection result on the other channels. Although the two algorithms differ completely in their implementation, they are closely related and matched to each other. Fig. 2 gives an overview of the complete detection strategy and implementation. The scheme serves as a guide through the paper and each block will be explained in the following sections. We will first explain the spike train type detection and then the oscillatory type detection.

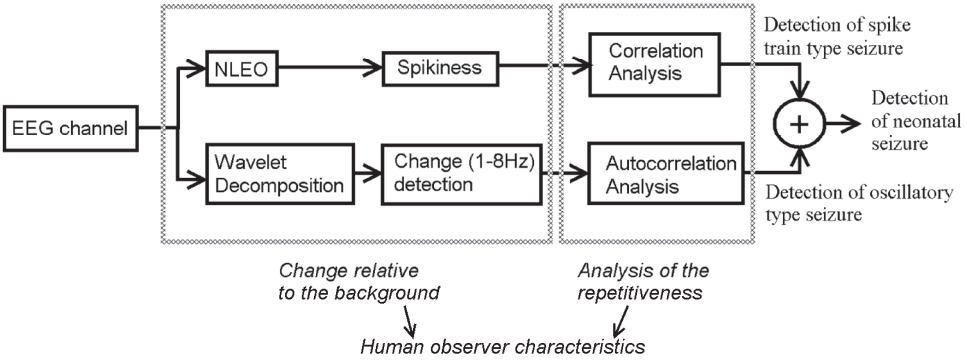

Fig 2. Schematic overview of the complete seizure detection algorithm. The upper track shows all steps of the spike train type detection, the lower track these of the oscillatory type detection. The complete seizure detection algorithm then consists of the sum of the two detection algorithms.

Detection algorithm for the spike train type

Our human observer approach indicated that the spike train algorithm must detect a *repetitive train* of *isolated spike like segments* of EEG. Accordingly, the algorithm consists of two steps. The first step isolates the spike-like segments and the second will analyze the similarity between these isolated segments.

a. Isolation of the spike like segments.

To detect the local presence of high frequency activity, we use a non-linear energy operator (NLEO) as proposed by Kaiser[18]. The NLEO represents the energy of a discrete time signal,

based on the physical energy of the signal producing system. In its discrete form, it is given by:

$$\psi_{kaiser}[x_{(n)}] = x_{(n)}^2 - x_{(n-1)}.x_{(n+1)} \tag{1}$$

With $x_{(n)}$ denoting the current sample of signal x, $x_{(n-1)}$ the first sample before sample n, etc. The key property of this operator can be derived by taking the following signals[19]:

$$x(n) = A.\cos(\omega_0 n + \vartheta)] \tag{2}$$

With A the amplitude, ω_0 the angular frequency and θ the phase,

$$\begin{aligned} x(n+1) &= A.\cos[\omega_0(n+1) + \vartheta)] \\ x(n-1) &= A.\cos[\omega_0(n-1) + \vartheta)] \end{aligned} \tag{3}$$

Using the following trigonometric identities:

$$\begin{aligned} &\cos(\alpha+\beta)\cos(\alpha-\beta) = \frac{1}{2}[\cos(2\alpha)+\cos(2\beta)], \\ &\cos(2\alpha) = 2\cos^2(\alpha) - 1 = 1 - 2\sin^2(\alpha) \end{aligned} \tag{4}$$

We become:

$$\psi_{kaiser}[x_{(n)}] \approx A^2 \sin^2(\omega_0 n) \tag{5}$$

This formula indicates that the output of the NLEO is proportional to the square of both the amplitude and the frequency ($\sin(\omega_0) \approx \omega_0$ for small ω_0). In this regard, the NLEO may be considered superior to other energy estimators that simply average the square of the signal and are independent of frequency. Because of these properties, the NLEO amplifies the high-frequency spikes of the spike train relative to the background EEG. A generalized version, which is more robust to noise, is given by Plotkin et al.[20]:

$$\begin{aligned} \psi[x_{(n)}] &= x_{(n-l)}.x_{(n-p)} - x_{(n-q)}.x_{(n-s)}, \\ l + p &= q + s \end{aligned} \tag{6}$$

As parameter settings, we used l=1, p=2, q=0, and s=3 (Agarwal et al, 1998)[21] to calculate the energy as locally as possible. Subsequently, on the output of the NLEO a moving average with a window size of 120 ms and shift of 1 sample was calculated. This value was chosen as a compromise for sensitivity between short spikes (<120 ms) and long spikes (>120 ms). The resulting signal was the smoothed, frequency-weighted energy of the EEG.

Next, this signal was divided into overlapping epochs of 5 s (overlap of 4 s). To each epoch, an adaptive threshold was applied that was defined as:

$$Threshold = 0,6.std(epoch) + q_3(epoch) \tag{7}$$

with 'std(…)' and 'q_3(…)' the standard deviation and 75th percentile of the epoch, respectively. The 75th percentile guarantees that only the highest energy parts of the signal are selected. The standard deviation adapts the threshold to the variability of the signal.

The human observer approach requires the spikes to be 'isolated' from the background activity. We implemented this requirement by calculating the spikiness of each high-energetic segment using:

$$Spikiness = \frac{max(segment)}{mean(background)} \tag{8}$$

The background is defined as the EEG with the length of the considered segment just before and after the spike. Only segments with a minimum length of 60ms and a spikiness higher than 7 were kept.

To summarize the segmentation, first those segments which are high-energetic with respect to the complete epoch are detected and next, the spikiness further reduces the number of segments to those which are high-energetic on a very local scale (isolated by comparatively low-energetic EEG).

b. Analysis of the correlation.

Following our human observer's approach, we now have to deal with the repetitive character of the seizures. In time domain processing, repetitiveness (or in this case: similarity between segments) can be estimated using correlation analysis[22]. To detect the occurrence of a repetitive pattern of segments, we developed a correlation scheme that will grow a set of highly correlated segments (Fig. 3). Such a set starts with just one segment and every detected segment of the segmentation step will be used as a starting point for the correlation scheme. In the first step, the cross-correlation of the first segment with the previous segment is calculated. If the maximum value of that cross-correlation is >0.8, that previous segment is added to the set. Next, the preceding segment is selected and the cross-correlation with the two segments of the set is calculated (step 2). If the mean of those two maximum cross-correlations is >0.8, that new segment is also added to set. In case of a repetitive signal, repetition of this process (step 3 etc.) leads to a set of segments which are highly correlated with each other. A detection of a seizure occurs when the number of correlated segments in the set is higher than 6.

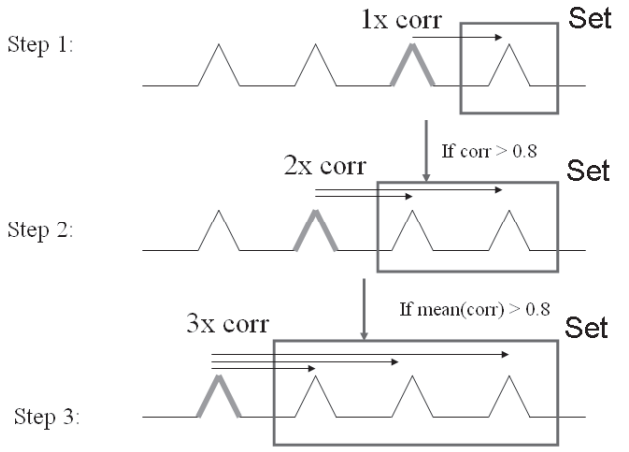

Fig 3. Analysis of the correlation between the segments. In case of a repetitive pattern, a set of highly correlated segments is grown. A spike train seizure is detected when at least 6 correlated segments are found.

An important parameter is when to stop the process as it is not efficient to search back for correlated segments indefinitely. This maximum time lag is dependent on the length of the first segment the process started with. Only segments which occur with a time lag less than 40 times the length of the first segment are checked.

By restricting the time lag based on the length of the first segment, the search is adapted to the length of the correlated segments the algorithm is searching for and thus adapted to the patient's EEG. In case the first segment is longer than 0.5 s, the maximum search back length is limited to 20 s. Due to this flexibility, the sensitivity of finding correlated spikes is the same for short as for longer segments (up to 0.5 s).

As this search for correlated segments is calculated for every segment detected in the segmentation step, several (possibly overlapping) sets of correlated spikes will be found in case of a spike-train type seizure. When the spikes of these sets are less than 20 s apart, they are grouped into a single detection.

The above process allows for some variation in the morphology of the spikes. The initial correlation must exceed 0.8, which implies a strong similarity. However, when a set starts

growing, the similarity may occasionally become less, as only the mean of the correlation with all segments of the set is used.

Fig. 4 shows the results of this algorithm. The grey shaded spikes are those that are detected as being part of a spike train type seizure. The segments with a rectangle around them are also detected by the segmentation step, but removed by the spikiness and correlation analysis and thus are not detected as being part of a spike train type seizure.

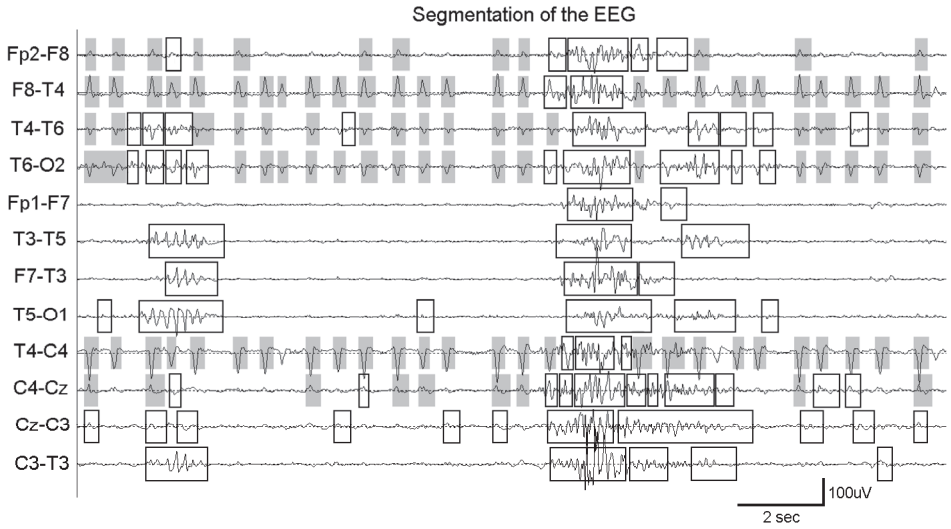

Fig 4. Example of spike train detection. All marked segments (grey shaded + marked by rectangle) are those detected by the segmentation step. After the spikiness operator and correlation analysis only the shaded segments remain. As a result, only those segments with a high correlation with previous segments (shaded) are kept and are detected as being part of a spike train type seizure.

Detection algorithm for the oscillatory type

A human observer seems to detect oscillatory type seizures as *continuous repetitive activity* that represents an *increase of low frequency activity* relative to the background. In time domain processing, the autocorrelation function is useful for finding a repeating pattern in a signal and, thus, for handling the repetitiveness of oscillator-type seizures. The autocorrelation is the cross-correlation of a signal with a delayed version of itself. Liu et al.[9] suggested a method based on the autocorrelation function to detect rhythmic discharges in the

neonatal EEG. Navakatikyan et al.[12] have shown that Liu's method displays a high sensitivity but also a high number of false positives. We therefore aimed to develop an autocorrelation-based analysis with a very low false positive rate. This algorithm consists of 2 steps. The first step detects an increase of low-frequency activity and the second analyzes the autocorrelation function of these increases.

a. Detection of an increase in low frequency content.

Every EEG channel is decomposed using the discrete wavelet transform (DWT). The signal is passed through two complementary filters and emerges as two signals. One is the low-frequency component, which is called the approximation, and the other is the high-frequency component or the detail. This process is repeated several times by successive filtering of the approximation. As a result, at each iteration (also called scale), a lower frequency component is extracted. As mother wavelet, we used a biorthogonal wavelet with decomposition order 3, as this wavelet matches the shape of the oscillatory activity we are looking for[23]. The oscillatory activity is most prominent in the δ (0.5-4 Hz) and θ (4-8 Hz) range of frequencies. Therefore, only those wavelet coefficients which correspond to scales of 1-2 Hz, 2-4 Hz and 4-8 Hz are used for further analysis.

Next, the derivative of each selected scale is calculated, and, the result is squared to make it positive and to enlarge differences. To detect significant changes in the resulting signal, a window with a length of 3 s is moved along the signal. Detection occurs if the third quartile of the signal in the window is higher than 2.5 times the third quartile of the previous 30 s of EEG. If a window is detected for a particular scale, the signal in that window represents a significant increase of activity in that specific wavelet sub-band, compared to the background. This activity is considered potential oscillatory seizure activity.

b. Autocorrelation analysis.

At this point, we have detected significant changes of activity in specific wavelet sub-bands of 1-8 Hz. What remains is the analysis of the repetitive aspect of the signal. As described above, our goal is to detect repetitive signals by means of the autocorrelation function. The detected windows in step 1 are divided into 5 s epochs (4 s overlap between epochs) and the autocorrelation function of the epochs is calculated. Fig. 5 shows two examples of epochs with their autocorrelation functions.

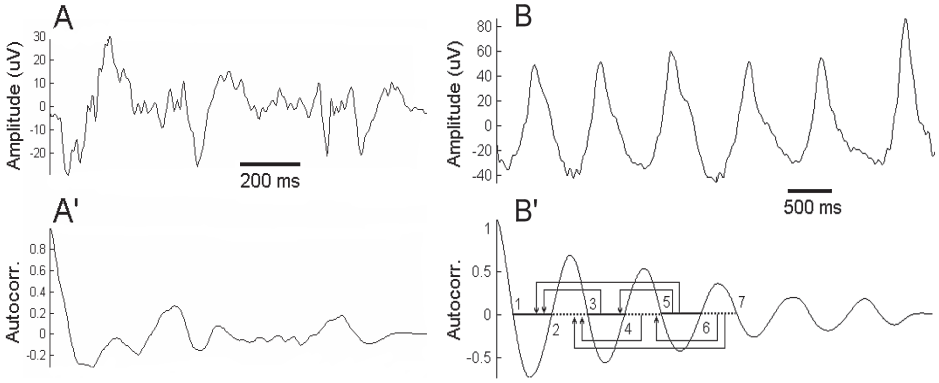

Fig 5. Difference between the autocorrelation functions (**A'** and **B'**) of a non-repetitive (**A**) and repetitive signal (**B**). The intervals between the zero crossings of the autocorrelation of a repetitive signal (**B'**) are regular. The arrows indicate which intervals are compared (each interval is used twice).

For our purpose, two features of the autocorrelation function are particularly useful. First, the autocorrelation of an oscillating signal is very symmetric around zero, whereas that of a random signal is not (Fig. 5). To exploit this difference, we calculate the skewness of the autocorrelation. Because of the symmetry, the skewness of the autocorrelation of an oscillating signal will be lower than that of a random signal. The second discriminatory feature compares the percentage difference of intervals between the zero-crossings of the autocorrelation (Fig 5B').

For the high-activity, low-frequency windows detected in step 1 to be classified as oscillatory seizure, their skewness must be lower than 0.4, and the mean of the interval differences between the zero crossings must be less than 6%.

Combination of the two detection algorithms

Roughly 50% of the seizures consist of a combination of spike train activity and oscillatory activity. In these cases, both detection algorithms are needed for a complete detection of the seizure. Fig. 6 shows a seizure that starts with oscillatory activity and ends with spike train activity. The oscillation-detection algorithm detects the beginning of the seizure but not the end (grey shaded areas). Conversely, the spike train detection algorithm detects the spike train at the end of the seizure, but not the beginning (areas with rectangle). There is a certain overlap between what is detected by both algorithms (channel Fp1-F3). When the seizure changes from oscillatory to spike train activity, the oscillation becomes spikier but the seizure is still continuous, resulting in detection by both algorithms.
When the results of both algorithms are combined, almost the entire seizure is detected.

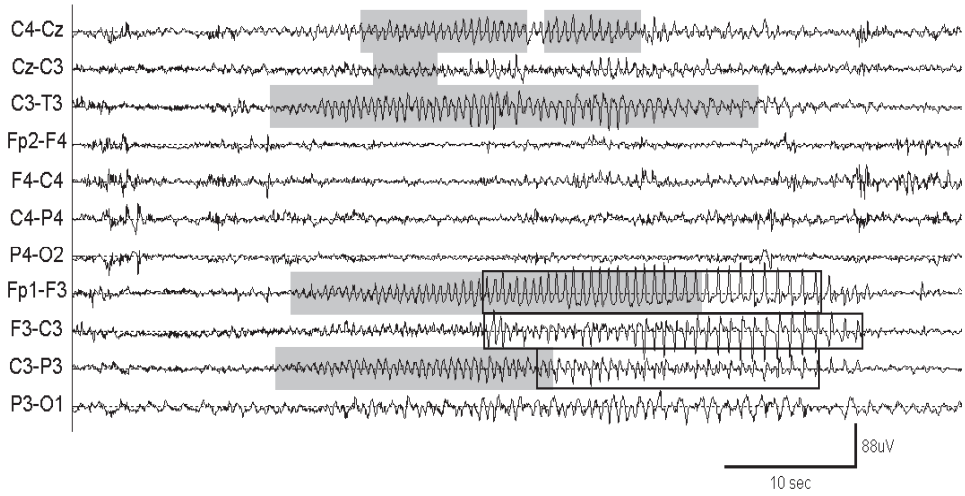

Fig 6. Result of the combination of both detection algorithms. The oscillation detection detects the beginning of the seizure (shaded areas) but not the last part (on channels Fp1-F3, F3-C3 and C3-P3). The spike train detection algorithm detects the last part of the seizure (marked by rectangle), which was not detected by the oscillation detection algorithm. The combination of both algorithms yields a complete detection. There is an overlap between the detection of both algorithms on channel Fp1-F3.

A neurologist reading the EEG usually classifies rhythmic EEG activity as a seizure if it is present for at least 10 s [24]. To mimic the human observer and to be able to detect these short seizures, we have set the minimum duration in our algorithm to 8 s.

Evaluation of the algorithm

Presenting the performance of a seizure detection algorithm is not straightforward. Different ways to measure the performance of an algorithm have been reported in the literature, leading to varying results, which cannot easily be compared. In addition, all these algorithms were tested on different datasets, which makes a comparison even more difficult.

We defined the sensitivity per patient (*SensPP*) as the percentage of the number of seizures marked by the neurologist that are detected.

$$SensPP = (SZ\det PP / SZtotPP).100 \tag{9}$$

with *SZtotPP* the number of seizures marked by the neurologist for a patient, and *SZdetPP* the number detected by the algorithm for that patient. A seizure was considered as detected when there was a temporal overlap between the marked seizure and the detection.

The total sensitivity for all patients is calculated using 2 methods. The first method simply averages all sensitivities per patient (*SensT_PP*). The second method (*SensT*) measures the percentage of seizures detected of all seizures present in the complete dataset. So, *SensT_PP* measures the expected sensitivity at the patient level and *SensT* at the seizure level. The importance of the difference is that in *SensT_PP,* a patient with only a few seizures is considered to be equally important as a patient with many seizures. *SensT*, on the other hand, regards all seizures as equally important regardless of the patient they occurred in.

The Positive Predictive Value (*PPV*) is defined as the percentage of detected events that match seizures.

$$PPV = (EVsz / EVtot).100 \tag{10}$$

with *EVtot* the total number of detected events and *EVsz* the total number of detected seizures. Occasionally, a single seizure was detected several times by the algorithm. All such events were combined into a single *EVsz* detection. The *PPV* is dependent of the a priori likelihood of seizures in the dataset, which makes it difficult to compare the *PPV* of different datasets. But nevertheless it is an interesting measure for the performance of the detector.

Another useful measure of performance is the number of false positive detections per hour (*fp/h*). This measure represents an important indicator of the practical usability of the

algorithm, because each FP implies that somebody in the NICU will have to check the patient and the raw EEG recording unnecessarily.

RESULTS

The results are given in Table 1.

The *SensT_PP* was 85%, *SensT* was 88%, as the algorithms detected 482 out of 550 seizures present in the dataset. The mean *PPV* was 75%. There were 143 false positive detections, or 0.66 *fp/h*. Of all false positives, 45% occurred in 2 of the 26 patients (patient 7 and 23). Closer analysis of these 2 patients revealed that both had low voltage EEG background activity (<15uV) and thus were very susceptible to artifacts. When these 2 patients were excluded from the analysis, the false positive rate dropped to 0.39 *fp/h*.

Table 1. Results of the combined algorithms. Patients 22 to 26 were seizure-free and thus no *SensPP* and *PPV* is given. (rec. len. is the recording length)

No.	*SensPP* (%)	*PPV* (%)	*fp/h*	rec. len. (h)
1	92	94	0,51	7,9
2	100	32	1,65	7,9
3	92	65	1,52	7,9
4	90	72	1,27	7,9
5	100	80	0,13	7,9
6	90	100	0,00	7,9
7	89	60	4,05	7,9
8	82	64	0,63	7,9
9	100	100	0,00	7,9
10	100	90	0,13	7,9
11	100	33	0,51	7,9
12	100	90	0,25	7,9
13	96	85	0,51	7,9
14	96	83	0,63	7,9
15	45	71	0,25	7,9
16	50	50	0,13	7,9
17	38	89	0,13	7,9
18	86	86	0,13	7,9
19	60	75	0,13	7,9
20	100	61	1,39	7,9
21	82	100	0,00	7,9
22	x	x	0,00	20
23	x	x	4,05	7,9
24	x	x	0,13	7,9
25	x	x	0,00	7,9
26	x	x	0,00	7,9

***SensT_PP*:**	**85%**
***SensT*:**	**88%**
***PPV*:**	**75%**
***fp/h*:**	**0,66**

DISCUSSION

In this paper, we introduce a new approach to detecting neonatal seizures. Our approach is based on two major characteristics of seizures (repetitiveness and a change relative to the background) that a human observer seems to employ. Although all seizures share these properties, their appearance can be very different (continuous versus discontinuous, low versus high frequency). Close observation, however, led to the identification of two types and, consequently, the development of two detection algorithms.

For this purpose, we use several concepts that were previously introduced in the literature (autocorrelation, NLEO, and wavelets), but we improve and combine them, while adding some of our own concepts. For example, just like Liu et al.[9], we make use of the autocorrelation – but we only use it to detect seizures with a continuous type of repetitiveness. Furthermore, instead of analyzing the autocorrelation of the complete EEG, we limit the analysis to those parts of the signal that represent an important increase of low-frequency activity relative to the background. The combination of these improvements results in a lower false positive rate compared to previously published algorithms without altering the sensitivity.

A novel property of the spike train detection algorithm is its ability to detect repetitive segments of EEG even when two neighboring seizure segments are interrupted by other EEG activity (Fig. 4). This adaptive, segmentation-based detection is much more flexible than analyzing a fixed epoch of EEG (like classifier-based approaches do). It is known that neonatal seizures are extremely variable in morphology and frequency[16,17], which makes it very difficult to develop a patient-independent algorithm. To deal with this variability, it is important to develop a data-driven algorithm that either derives its thresholds from the data or uses parameters that are independent of the variability. For instance, in our approach, the thresholds for the segmentation of the EEG in the spike train detection are derived from the data (‘q3’ and ‘std’ of the amplitude). Similarly, in the oscillation detection, the algorithm references to the background EEG. The remaining fixed thresholds are the correlation between the segments and the features derived from the autocorrelation. However, these parameters are independent of the variability of the seizures, because they only exploit the repetitiveness of the seizures and are independent of the actual seizure shape.

The sensitivity of the combined algorithms was good (*SensT_PP* 85%, *SensT* 88%) at a practically acceptable false positive rate of 0.66 *fp/h* and mean *PPV* of 75%. For comparison, the algorithm by Navakatikyan et al.[12] reported sensitivities of 83-95%. *PPV* of 48-77% and 2 *fp/h*. The algorithms of Gotman[10] and Liu[25] were also evaluated by Navakatikyan et al[12]. In their evaluation, the sensitivity of the Gotman algorithm ranged between 39.3 and 87.9%, and for the Liu algorithm between 97.9 and 99.2%. However, the false positive rate for the Gotman algorithm ranged from 7.4 to 10.4 *fp/h*, and an even higher 15.7 *fp/h* for the Liu algorithm. Faul et al.[26] have also evaluated the performance of the Liu and Gotman algorithms. They reported a sensitivity of 62.5% for the Gotman algorithm and 42.9% for the Liu algorithm. Unfortunately, no *fp/h* measure was given.

In general, for both algorithms, long, high-amplitude seizures are rarely missed. The algorithms' sensitivity is lowest for subtle, short seizures which lack repetitiveness (arrhythmic) (Fig. 7). Finally, Fig. 8 provides a few examples of false positive detections of extra-cerebral activity of biological origin; Fig. 9 shows some false positives caused by non-biological external sources. The ECG artefact was very dominant and present throughout the recording in a few patients. To deal with this artefact we developed an ECG reduction algorithm based on Independent Component Analysis (ICA) and applied it to the EEG before the seizure detection algorithm was run (a detailed description of this algorithm is beyond the scope of this paper). This greatly improved the false positive rate, but in spite of this step, the ECG artefact remained responsible for some false positives.

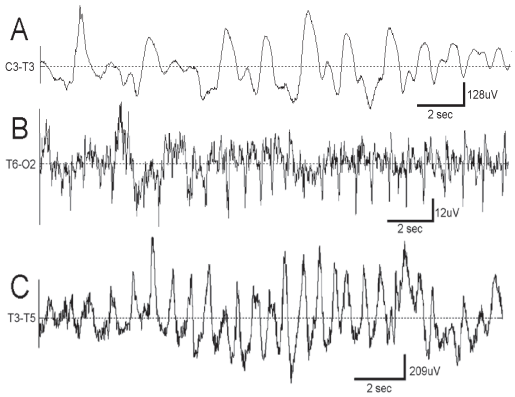

Fig 7. Examples of missed seizures. **A**: high-amplitude, arrhythmic oscillatory seizure, **B**: short, low-amplitude spike train (complete seizure is shown). **C**: short, focal, high-amplitude seizure (complete seizure is shown).

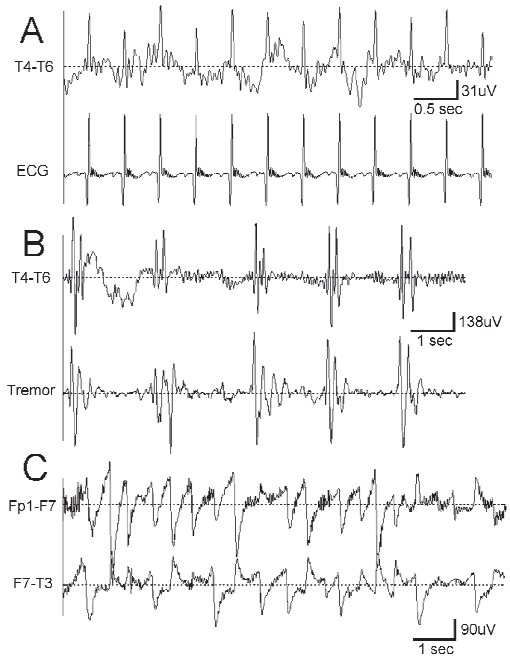

Fig 8. Examples of false detections of biological origin. **A**: ECG artefact, **B**: tremor artefact, **C**: pathological fast eye movements (nystagmus).

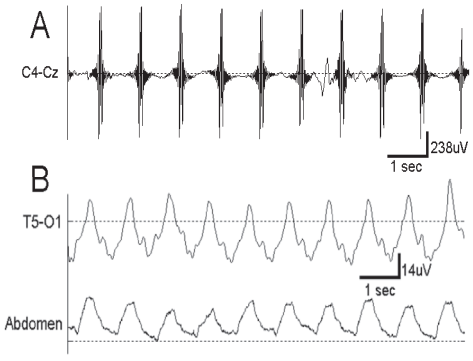

Fig 9. Examples of false positive detections of non-biological origin. **A**: high-frequency artifact, due to the heating of the bed, **B**: ventilation artifact, as shown by the high correlation with a sensor on the abdomen.

During validation of our methodology, we regularly encountered rhythmic activity in the EEG, detected by the algorithm. The origin of that EEG activity was not always clear, even for the neurologist, by only using the information from the scalp electrodes. In those cases, additional information from the simultaneously recorded polygraphic channels of ECG, respiration, movement sensor or even video were needed to establish that the activity present in the EEG was cerebral in nature or caused by an extra-cerebral source. To decrease the false positive rate of our algorithm further, these signals will have to be included in the analysis, as it is impossible to correctly classify them based on EEG analysis alone. At this point, we have only included the simultaneously recorded ECG in our analysis, by performing an ECG reduction on the data before the seizure detection algorithm was run.

The present parameter settings are for offline analysis and to obtain maximum sensitivity at an acceptable false positive rate. These values are based on expert estimation and trial and error. It is nearly impossible to estimate the parameters in a more systematic approach (e.g. using ROC-curves). Because there are many parameters, which are dependent of each other, we would need an almost infinite amount of ROC-curves to define the optimum parameter values. This optimum would also only be optimal for this particular dataset. We think our approach proves the robustness of the algorithm as it is able to perform without extreme optimization of the parameters. In a monitoring environment it might be desirable to obtain a lower false positive rate at the cost of a lower sensitivity. For the spike train type detection algorithm this can be achieved by increasing the threshold on the correlation between the detected segments. For the oscillatory type detection, the threshold on the skewness and the allowed percentage difference between the zero crossings may be decreased. By tuning these parameters we believe the algorithm to be suitable for online monitoring with sufficient sensitivity and practical false positive rate. The dataset on which the algorithm was tested consisted only of full-term neonates with perinatal asphyxia. In the future we would like to test it on preterm neonates. Nevertheless, the fact that our algorithm demonstrates a high sensitivity in the presence of all possible types of background activity suggests that it may also perform well on other kinds of EEG, including preterm EEG. The dataset used in this study comes from one center only. To further corroborate these results we need to test it on multi-center datasets. This is what we are planning to do in the near future.

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3.2 Validation of a new automated neonatal seizure detection system: a clinician's perspective

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Submitted for publication

ABSTRACT

***Aim*:** To validate an improved automated neonatal seizure detection algorithm (*NeoGuard*) in a large and independent data set, and to evaluate its performance in relation to EEG background.

Methods: We classified EEG background into eight grades based on evolution of discontinuity and presence of sleep-wake cycles. Patients were further sub-classified into two; gp**I**: those with mild to moderate (grades 1-5) and gp**II**: severe (grades 6-8) abnormalities. Seizures were categorized as definite and dubious. Seizure characteristics were compared between the two EEG groups. The algorithm was tested on 756 hours of EEG monitoring data from 24 consecutive neonates (median 25h per patient) with encephalopathy and recorded seizures. No selection was made regarding quality of EEG or presence of artefacts.

Results: Seizure amplitude showed a significant negative correlation with worsening EEG background. Seizure frequency and percentage of arrhythmic seizures showed a tendency to increase, while the seizure duration showed a tendency to decrease with worsening EEG grade, though the results were not statistically significant. After excluding 4 patients with persistent, severely abnormal EEG background, and predominantly having dubious seizures characterized by low amplitude arrhythmic discharges, the algorithm showed a median sensitivity per patient of 86.9%, positive predictive value (*PPV*) of 89.5% and false positive rate of 0.28/h (1263/1538 seizures detected, total sensitivity 82.1%). Sensitivity tended to be better for patients in gp**I**.

Conclusion: The algorithm detects neonatal seizures well, has a good *PPV*, and is suited for long-term EEG monitoring. The change in electrographic characteristics in relation to EEG background tends to affect the performance of automated seizure detection.

INTRODUCTION

Neonatal seizures occur commonly in the neonatal intensive care unit (NICU) and usually suggest serious neurological dysfunction[1]. The majority of the seizures occurring in encephalopathic neonates are subclinical, being detected only by continuous EEG monitoring[2]. However, detection and treatment of neonatal seizures is hampered by the limited access to EEG monitoring. It is an expensive and labor-intensive technique and its interpretation requires specialized training. Even in the few NICUs where it is available, expert evaluation of the EEG by a neurologist/clinical neurophysiologist is not available around-the clock, thus limiting its usefulness. In the past years, many NICUs have adopted amplitude integrated EEG (aEEG), which displays time-compressed and asymmetric band-pass filtered data from a single EEG channel. However, the limited spatial and temporal sampling results in loss of EEG information and also the results of visual interpretation are variable[3-5]. Improvement of neonatal EEG monitoring can be achieved by the development of a reliable automated seizure detection method with a fair degree of sensitivity and low false positive detection rates[6-10]. Presently, there is no commercially available multi-channel EEG-based neonatal seizure detection algorithm that is widely accepted for clinical use. Another reason that EEG monitoring coupled with automated neonatal seizure detection is not widely introduced yet is the lack of convincing evidence that treatment of subclinical (electrographic) neonatal seizures with antiepileptic drugs (AED) improves clinical outcome. It is known that in neonatal encephalopathy, electrographic seizures occur mostly in association with an abnormal EEG background [11,12]. Abnormality of background EEG reflects the severity of underlying encephalopathy[13] and is a strong predictor of outcome[14-16], especially after perinatal asphyxia. Normal/mildly abnormal EEG strongly predicts normal outcomes while a severely abnormal EEG at 48 hours post partum (PP) predicts poor outcome[17]. Electrographic seizure characteristics like measures of seizure burden[18,19], number of seizure foci [16], persistence for ≥48 hours [18] etc. have also been reported to be associated with poor outcome.

To be clinically relevant, the detection and interpretation of neonatal seizures should be done in the context of EEG background activity. Recently, we have developed an automated neonatal seizure detection system that mimics the human interpreter reading the EEG, with promising results in an initial study[20]. We have further improved it, especially in rejecting artefacts[21]. Aim of the present study was to test this improved algorithm (called *NeoGuard*) in a new and much larger EEG data set. Secondary aim was to assess the performance of the algorithm in detecting electrographic seizures occurring on varying degrees of EEG background abnormality, which has not been done previously.

PATIENTS AND METHODS

EEG data from 45 consecutive newborns with presumed perinatal asphyxia, who underwent video-EEG monitoring for ≥24 hours (h), between March 2003 and August 2007 as part of an ongoing study, and had seizures recorded during the monitoring formed the data base. The inclusion criteria for EEG monitoring were: clinical features of encephalopathy, and having at least one of the following features of birth asphyxia: a) arterial pH of umbilical cord blood ≤7.1, b) Apgar score ≤5 at 5 minutes, and c) high clinical suspicion (like fetal distress, umbilical cord prolapse, difficult labor, or a history of convulsions). Three of these neonates (patient nos. 1,4 & 5) were subsequently found to have arterial ischemic stroke on MRI. All the patients except two (patient nos. 17&19) with gestational ages of 30 and 35 weeks) were born at term. MRI of the brain was available in 21, done according to an asphyxia protocol and scored by one neuroradiologist[22]. Babies with congenital cardiac abnormalities and multiple congenital anomalies, were excluded. Twenty-one neonates with recorded seizures, who were described in our previous article describing the seizure detection algorithm, were also excluded, leading to a large new set of 'unseen' monitoring data from 24 newborns for the algorithm.

***EEG registrations*:** Digital video-EEG with polygraphy, started mostly ≤24 h PP, was registered continuously for 1-3 days using a Nervus™ monitor (Taugagreining hf, Reykjavik, Iceland). Seventeen scalp electrodes were applied according to the full 10-20

International System[23] in all except two patients. A restricted 10-20 system with 13 electrodes (Fp1-2, C3-4, Cz, F7-8, T3-4, T5-6, O1-2) was used in one, while in the other, nine electrodes (Fp1-2, C3-4, Cz, T3-4, O1-2) were used. Polygraphy included ECG, respiration, electro-oculogram(EOG), chin EMG and limb movements. EEG sampling frequency was 256 Hz. Band-pass filter was 0.3 -70 Hz.

EEGs were interpreted by a clinical neurophysiologist as part of patient care and this information was used to make treatment decisions. All patients in this study received loading doses of phenobarbitone (20mg/kg). In patients with persistent electrographic seizures, intravenous midazolam was given as a loading dose of 0.1mg/kg, followed by maintenance doses of 0.1- 0.5 mg/kg/h. Babies with seizures refractory to midazolam received intravenous lidocaine, 2mg/kg, followed by a maintenance dose of 6mg/kg/hour, which was tapered off over 30 h. The AED treatment was according to a departmental protocol with every next step taken when no effect on electrographic seizures was seen within one to two hours, and is comparable to the ones widely used in European NICUs[24]. The study had the approval of the Erasmus MC Medical Ethical Review Board.

Scoring EEG background: We used an eight grade system (***Appendix***), derived from literature[15,17,25,26] and modified based on our clinical experience. This scoring system emphasizes discontinuity (voltage and length of the discontinuous periods) and its evolution over 24 h, presence of variability, reactivity and SWC. Severity of discontinuity is fairly easy to assess and is a robust predictive parameter of outcome[27]. Discontinuity was defined as voltage attenuation (< 25μV for mild and <10 μV for severe) in at least 50% of the EEG channels, lasting for ≥5 seconds. To score discontinuity, the most frequently occurring measure of the amplitude and duration of the discontinuous periods were used. EEGs were blindly scored by a clinical neurophysiologist at the time of recording and for this study, were reviewed again by the clinical neurophysiologist (PJC) together with a neonatologist (RMS). Consensus was reached in all cases. We omitted parameters such as frequency content and duration of the EEG bursts, frequency of sporadic or rhythmic sharp waves within and outside the bursts, because we wanted the classification to be readily applicable at the bedside. We also avoided the term burst-suppression, as this has been defined variably in neonates[27].

Electrographic seizures: We defined as 'definite seizures', electrographic discharges that showed a clear variation from background activity, displaying a repetitive pattern of oscillations or sharp waves or a mixture of both (Fig 1A & B), lasting ≥10 seconds, with evolution in amplitude and frequency over time [20,28,29]. We also classified discharges as 'dubious seizures' a) runs of sharp waves /oscillations or a mixture of both occurring arrhythmically (with marked variability in the interval and morphology between individual complexes in a seizure discharge for the major part of its duration) or b) rhythmic discharges of shorter (<10 sec) duration or periodically occurring sharp waves or mixed patterns. It was difficult to identify the onset and offset of such discharges and sometimes difficult to clearly identify them as a variation from ongoing EEG background (Fig 2B & 3). We chose to group them under 'seizures' as they were seen to recur paroxysmally during the monitoring. Inter-rater agreement among two experienced clinical neurophysiologists (PJC and GHV) for scoring of seizure occurrence in one hour segments of EEGs from 10 patients from another data set, was 73% [20], with a *Kappa* value of 0.4. Subsequently, these data were reviewed jointly by both and the scored seizures were agreed upon by consensus. For this study, the EEGs were reviewed in their entirety by a neurologist/clinical neurophysiologist (PJC), blinded to the clinical and MRI results, and the onset, location, spread, frequency and rhythmicity of the electrographic seizures were documented.

Fig 1.A. Left central-temporal-parietal seizure expressing mixed (sharp waves and oscillations) pattern in patient no.8. It is difficult to identify the seizure in the aEEG trend shown at the top, probably due to contamination of EEG by muscle artifacts. **B**. Detection of the seizure by the algorithm after automated removal of artefacts (timebase 30 sec/page, the first 12 seconds corresponds to EEG segment shown in **A**)

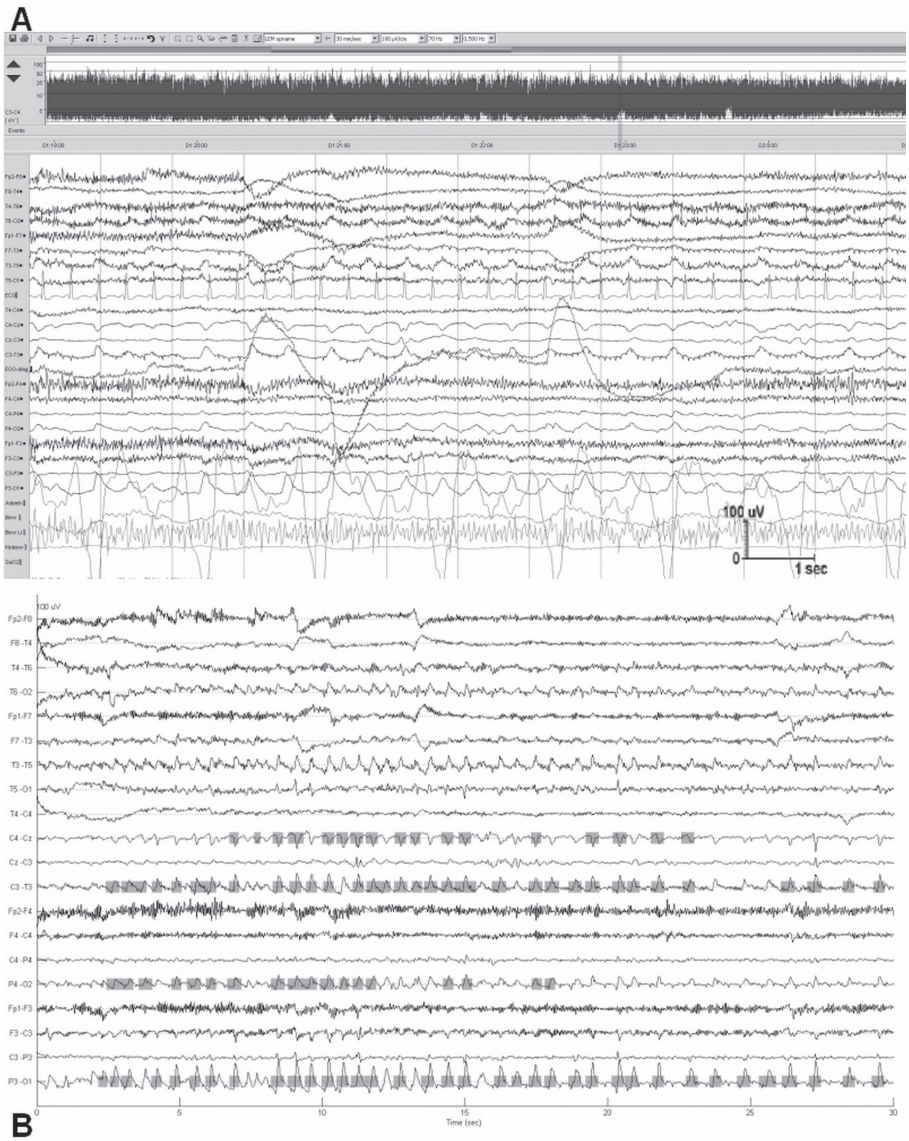


Fig 2. **A**. Seizure in patient no.5 with right middle cerebral artery territory stroke, showing rhythmic sharp waves and mixed patterns over the central-parietal regions, detected by the algorithm. **B**. Dubious seizures in the same patient, characterized by brief rhythmic discharges and periodic sharp waves, occurring over the same regions. These shorter discharges were variably detected by the algorithm.

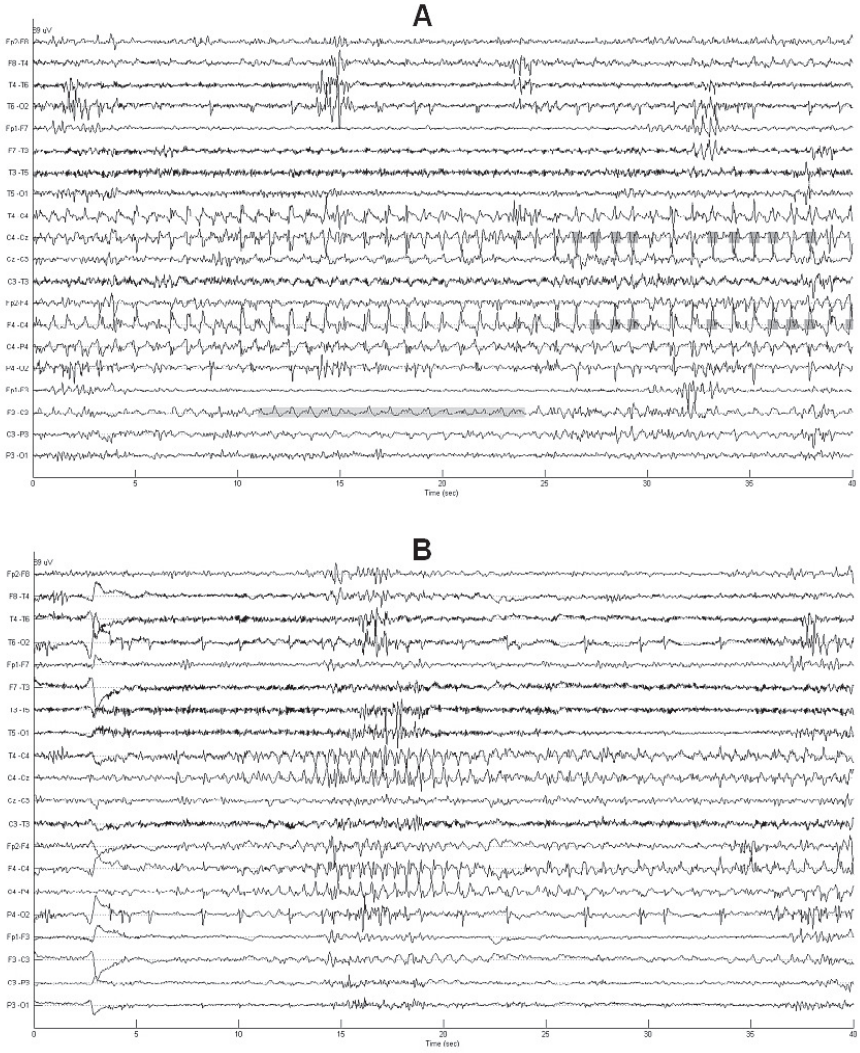
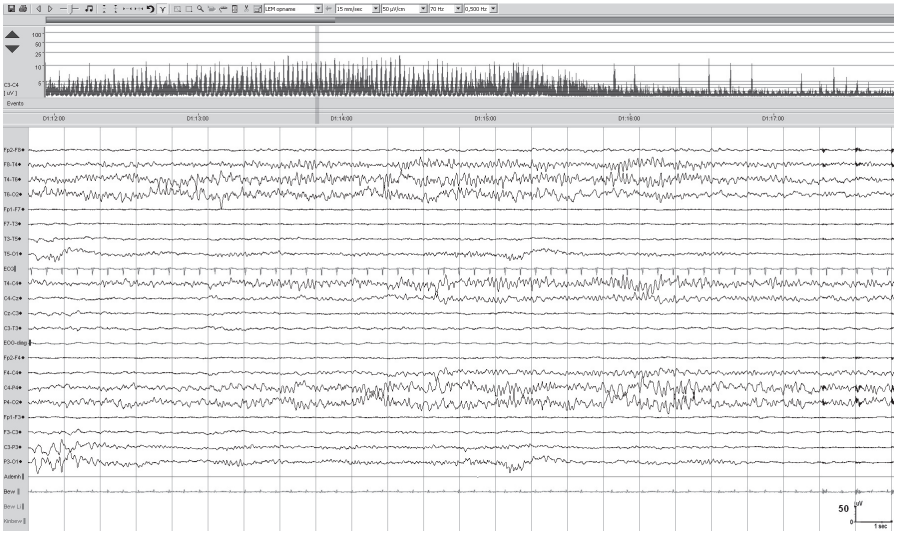


Fig 3. Dubious seizure. Arrhythmic low amplitude discharges occurring over the right temporal-parietal regions (a paroxysmal pattern is well seen in the aEEG trend at the top), not detected by the algorithm, associated with very severe abnormality (isoelectric or persistent low voltage) of EEG background, in patient no.12.



Seizure detection algorithm: The technical details of this algorithm have been discussed in detail in a previous article [20]. Briefly, this consists of two detectors running in parallel: a) a spike-train detector that detects high-energy segments of the EEG and analyzes the correlation between them and b) oscillatory seizure detector that detects increase in low frequency activity (1-8Hz) with high autocorrelation. This algorithm has been further improved, especially in artefact reduction. We identified the three most important types of artefacts hindering seizure detection, namely: ECG spike artefacts, blood vessel pulsation and respiration artefacts. An algorithm has been developed [21]to remove these three artefacts from the EEG using the input from the simultaneously recorded polygraphy channels (ECG and respiration movement). The algorithm searches for activity in the EEG channels that had a high correlation with activity in the polygraphy channels. Only after automatically removing such activity from the EEG channels, is the seizure detection algorithm applied. The algorithm runs on the Matlab™ (The MathWorks, Natick, MA, USA) platform. It can run online during bedside registration of EEG. For this study, all the EEG monitoring data were converted to European Data Format and the algorithm was run offline independently by two of the authors (PJC and WD). No selection of EEG data based on presence of artefacts was made. The detections were reviewed independently and were compared to the visual scoring done by the clinical neurophysiologist. For each patient, a list of true positives, missed detections (false negatives) and false positives were made. As the algorithm provides an output of event-based detection (not epoch-based, as reported by studies evaluating shorter segments of EEG), we do not have a value for true negatives. Missed seizures as well as false positives were jointly reviewed. Consensus was reached in all cases. We calculated the sensitivity per patient (*SensPP*), the total sensitivity(*SensT_PP*) and positive predictive value (*PPV*) as described previously[20]. We also looked at the number of false positive detections per hour (*Fp/h*) as well as the cumulative duration (in seconds) of *Fp* detections.

Statistical analysis: Since there were not enough number of patients for a three-way classification (mild, moderate and severe EEG background abnormalities), dichotomous classification of patients was done [gp**I**: normal to moderately severe (grades 0-5) and gp**II**: severely abnormal (grades 6-8)] for comparing the electrographic characteristics of

seizures as well as the performance of the seizure detection algorithm. Comparison of two groups was done using Mann-Whitney U test or the Fisher's exact test. A *p* value of <0.05 was considered significant.

RESULTS

A total of 756 h of EEG data was studied. Median duration of EEG recording was 25 h (range 17 to 78). On an average, the offline running of the algorithm on EEG data of 24 h (about 1 GB of data) from a patient on a laptop computer with a 2.26GHz Core™ 2 Duo processor (Intel Corporation, Santa Clara, CA, USA), was done in about 12 h. The algorithm gives an output of the number of events detected, the position in time where the event was detected and the duration of the detected events. The detections can also be visually reviewed (Fig 1B). A total of 2103 seizures were scored visually (median 67 per patient, range 7-236).

Ten patients had mild to moderate abnormalities of EEG background (gp**I**, grades 1-5), while 14 had severe abnormalities (gp**II**, grades 6-8). MRI scans were not done in three neonates in gp**II**. In the 21 newborns who had an MRI scan of the brain, lesions of basal ganglia and thalamus were significantly increased in patients in EEG gp**II**, when compared to gp**I** (9/11 vs 2/10, p=0.009, Fisher's exact test). Follow-up data for at least two years was available in all patients except one in gp**I**, who had a normal MRI. Severely abnormal outcomes (death or severe handicap) were significantly increased in patients in EEG gp**II**, with 10 patients dying and 4 developing severe handicap (gp**II** vs gp**I**, 14/14 vs 2/9, p<0.001, Fisher's exact test).

Table 1A: Results of visual scoring of EEG and automated seizure detection in patients with mild to moderate abnormalities (grades 1-5) of EEG background

No.	*EEG gr*	*Morphol*	*Freq*	*Amp*	*Dur*	*Arrhy%*	*Sz detec*	*Sens*	*Fp*	*PPV*	*Fp/h*	*durFp*
1	1	mixed, osci	2	60	25	0	52/53	98	21	84	0.88	234
2	2	shw	1	50	21	0	10/18	56	1	92	0.04	17
3	5	shw	1	35	86	80	28/48	58	19	60	0.42	512
4	5	shw	1	50	49	8	30/34	88	34	47	2	1947
5	1	mixed	1	150	66	11	56/63	89	0	100	0	0
6	2	osc, shw	2	100	174	15	12/13	92	4	75	0.17	99
7	2	shw	5	50	40	6	104/109	95	0	100	0	0
8	2	mixed	2	60	108	0	8/8	100	0	100	0	0
9	2	mixed, osc, shw	7	100	63	4	93/98	95	0	100	0	0
10	4	mixed, shw	1	65	18	100	6/7	86	6	50	0.26	113

Table 1B: Results of visual scoring of EEG and automated seizure detection in patients with severe abnormalities (grades 6-8) of EEG background

No.	*EEG gr*	*Morphol*	*Freq*	*Amp*	*Dur*	*Arrhy %*	*Sz detec*	*Sens*	*Fp*	*PPV*	*Fp/h*	*dur Fp*
11	8	mixed	1	150	37	0	110/112	98	6	95	0.29	98
12	8	mixed, osc	8	10	20	100	0/210	0	45	0	0.09	45
13	8	osc	4	30	20	100	1/70	1	8	11	0.33	180
14	6	shw	2	25	106	0	47/50	94	3	94	0.13	99
15	6	mixed	3	35	35	60	30/72	42	7	81	0.17	129
16	8	osc	1	150	32	0	18/33	55	15	55	0.65	231
17	8	mixed, osc	8	50	89	44	95/113	84	1	99	0.04	42
18	8	shw	5	20	33	0	169/200	97	10	94	0.42	596
19	6	mixed, osc	8	25	20	65	14/44	32	12	54	0.41	347
20*	8	mixed, osc	3	25	30	0	10/27	37	69	13	3.45	3588
21	8	mixed, shw, osc	6	25	25	100	12/156	7	0	100	0	0
22	8	mixed, shw	3	30	75	10	170/200	85	10	94	0.42	200
23	7	mixed, shw	7	15	20	90	9/129	7	80	10	3.2	4603
24	8	mixed, shw, osc	1	50	64	30	201/236	85	31	87	0.47	1026

EEG gr: EEG background grade, *Morphol*: seizure morphology (*osc*: oscillations, *shw*: sharp waves, mixed: mixed patterns) , *Freq*: seizure frequency(Hz), *Amp*: amplitude (μV), *Dur*: duration (sec), *Arrhy %*: percentage of arrhythmic seizures, *Sz detec*: detected no. of seizures, *Sens*: sensitivity per patient, *Fp*: no. of false positive detections. *PPV*:positive predictive value, *Fp/h*: false positives per hour, *durFp*: total duration of false positives(sec). Patient nos. 12, 13, 21 and 23, highlighted in grey, with persistent, severely abnormal EEG background and predominantly low amplitude arrhythmic discharges (dubious seizures) were excluded from the final analysis. Values reported are means.

Electrographic characteristics that may influence automated seizure detection:

For the whole group, the median values (range) were: seizure frequency 2.3Hz (1-8), amplitude 50μV (10-150), duration 36 sec (18-174) and percentage of arrhythmic seizures 11% (0-100). EEG background abnormality (grades 1-8) as well as the seizure characteristics like mean amplitude, frequency, duration, morphology and the percentage of arrhythmic seizures expressed in each patient is shown in Tables 1A & B. There was a significant negative correlation between the seizure amplitude and the EEG background grade (Spearman's *rho*= -.475, *p*=0.019). There were trends for increasing expression of arrhythmic seizures, increase in seizure frequency and decrease in individual seizure duration with increasing severity of EEG background abnormality, though the results did not reach statistical significance (results not shown). Increasing expression of arrhythmic seizures (expressed as a percentage of total number of seizures) tended to be associated with lower seizure amplitude (Fig 4). In patients in EEG gp**II,** the total number of seizures expressed was significantly increased while the amplitude of the seizures was significantly decreased (Table 2 & Fig 5). There was a tendency for patients in this group to express more seizures with a higher firing frequency.

Table 2. Comparison of patients with mild to moderate (gp I, grades 1-5,) and severe (gp II, grades 6-8) abnormalities of EEG background activity.

Characteristic	***gp I(n=10)***	***gp II(n=14)***	***p value***[†]
Duration EEG(h)	25 (17-78)	24(20-66)	0.75
Sz number	41 (7-109)	112(27-236)	0.008
Sz duration (sec)	56(18-174)	33(20-106)	0.25
Sz frequency(Hz)	1.25(1-7)	3(1-8)	0.052
Sz amplitude(μV)	60(35-150)	30(15-150)	0.007
Arrhyth sz %	7(0-100)	30(0-100)	0.44
Sensitivity per patient(n=24) (n=20)*	90.6(56-100) 90.6(56-100)	55((1-98) 84.6(32-98)	0.015 0.104
PPV(n=24) (n=20)*	88(47-100) 88(47-100)	86.6(10-100) 90.3(13-99)	0.38 0.595
Fp/h(n=24) (n=20)*	0.11(0-2) 0.11(0-2)	0.41(0-3.45) 0.42(0.04-3.45)	0.158 0.128

h:hours, *Sz*: seizure, *Arrhyth sz %*: percentage of seizures that were arrhythmic. * : for the final analysis, 4 patients in gp II with persistent severe abnormality of EEG background and having predominantly dubious seizures were excluded. Values reported are medians (range). †: Mann-Whitney test.

Fig 4: Scatter plot showing a tendency for increasing percentage of arrhythmic seizures to be associated with lower seizure amplitudes (Spearman's *rho*= -0.34, *p*=0.11). Line of fit with 95% CI is shown.

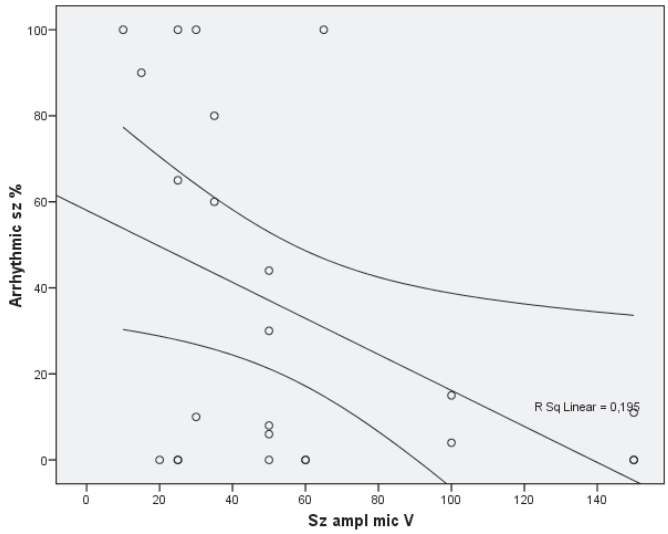
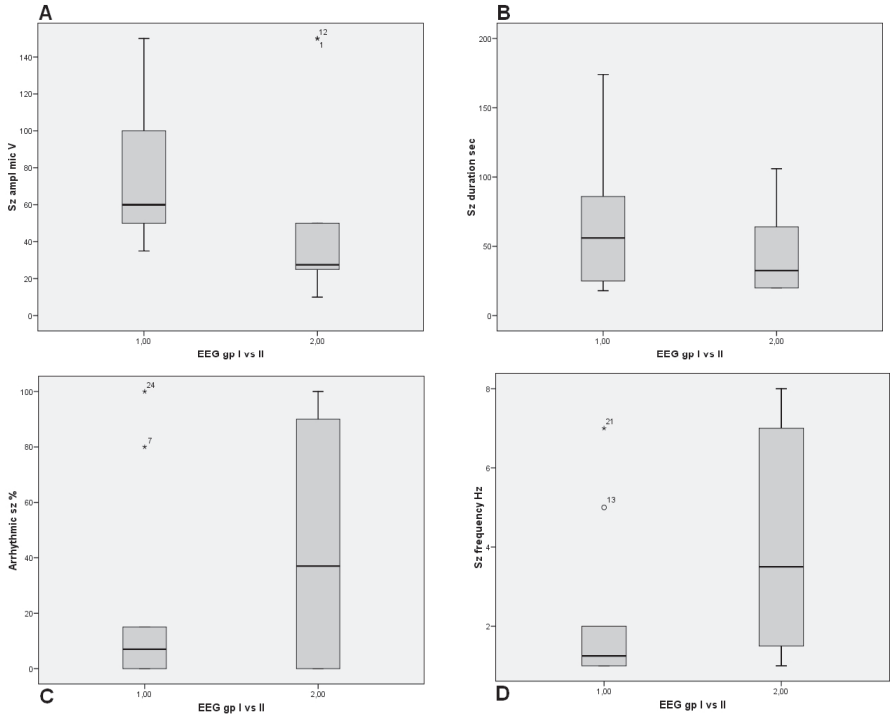


Fig 5. Box plots comparing various ictal parameters (**A**: amplitude in μV, **B**: duration in sec, **C**: percentage of arrhythmic seizures and **D**: seizure frequency in Hz) of individual seizures in patients with mild to moderate (gp**I**=grades 1-5, n=10) and severe (gp**II**=grades 6-8, n=14) abnormalities of EEG background. Only the amplitude of seizures was significantly different between the groups (Mann-Whitney U=24.5, p=0.007).



Dubious seizures: Four patients in gp**II** had predominantly (90-100%) 'dubious' seizures, with low amplitude arrhythmic seizures expressing oscillatory morphology or mixed patterns (Fig 3). One patient in gp**I**, with a middle cerebral artery territory stroke had definite seizures, and in addition, 'dubious' seizures consisting of short runs of rhythmic sharp waves (Fig 2B) in the same location [30] and at times in a periodic manner (periodic lateralized epileptiform discharges or PLEDs).

***Performance of the algorithm*:** The results are given in Tables 1 & 2. When both definite and dubious seizures were considered together, 1285/2103 seizures were detected, giving a *SensT* of 61.1 %. The average of all sensitivities of the 24 patients (*SensT_PP*) was 65.9% (median 85.1%, range 0-100), with a mean *PPV* of 73.7% (median 86.6%, range 10-100). In four patients with severely abnormal EEG background activity (gp**II**) and predominantly (>90%) dubious seizures, the algorithm performed very poorly (*SensPP* 0-7%). As it was doubtful whether this recurring paroxysmal activity (Fig 3) constituted genuine seizures, we excluded these patients. In the remaining 20 patients, the algorithm showed a *SensPP* of 86.9%, *PPV* of 89.5% and *Fp/h* of 0.28/h (totaling 643h of EEG data, 1263/1538 seizures detected, *SensT* 82.1%).

***Comparison of seizure detection in patients in EEG gpI vs gpII*:** The algorithm showed a tendency for increased sensitivity in patients with mild to moderate abnormalities of EEG background (gp**I**) (after excluding patients with 'dubious' seizures) but the difference was not statistically significant (Table 2).

Electrographic characteristics of missed seizures: The algorithm showed a low sensitivity (32 to 55%) in 4 patients in gp**II**. In patient nos. 15 and 19, low amplitude arrhythmic electrographic discharges constituted 60-65% of the seizures. In patient nos. 16 and 20, the missed seizures were of lower amplitude (20 to 35μV) and shorter (15 to 20 sec) but similar in morphology and location to the detected seizures.

In 2 patients in gp**I**, a relatively lower sensitivity (56 and 58%) was seen. In patient no.2, low amplitude seizures (20-30 μV) were missed while in patient no.3 with a moderately severe EEG background abnormality (grade 5), low amplitude arrhythmic seizures constituted 80% of the seizures.

Artefacts: The number of false positive detections per patient is shown in Tables 1A & B. Both biological (pulsation, respiratory movements, eye movements including nystagmus

and tremor) and non-biological (electrode, ventilator and bed warming) artefacts caused false positive detections. Two patients showed a high number of artefacts. In one (patient no. 23), these were related to head movements associated with respiration, involving O1 or O2 electrodes. In the other, (patient no. 20), majority of the false positives were related to artefacts involving the C3 electrode.

DISCUSSION

Performance of the algorithm: In this new and extensive data set, the neonatal seizure detection algorithm (*NeoGuard*) showed a good sensitivity and *PPV*, suggesting its suitability for long-term EEG monitoring in a NICU setting. No patients with seizures were missed. Though we did not compare the results of the algorithm with visual detection using aEEG, a potential advantage of the automated detection is its high *PPV* (89.5%). Use of aEEG alone (1 or 2 channels) has been reported to show a low sensitivity (27-56%) and low inter-observer agreement (0.3) even among expert users[31].

Inter-observer agreement for visual scoring of seizures: It is not surprising that inter-observer agreement was only fair (K=0.4) between two expert raters for visual scoring of seizure occurrence. Correlation of human experts for scoring seizure data from epilepsy patients has identified that both very short and long seizures, high seizure rate per hour and ambiguous offsets can result in poor correlation[32]. As opposed to patients with chronic epilepsy, such situations are encountered more frequently in neonatal seizures occurring in encephalopathic infants in the NICU, making a compelling argument for the use of automated seizure detection.

Missed seizures: Seizures with very low amplitude and short duration were missed by the algorithm and this problem has also been reported by other authors[33]. We also found the automatic detection of arrhythmic seizures (predominantly oscillations or mixed patterns) of low amplitude to be poor, while arrhythmic seizures with sharp wave morphology were well-detected. Seizures with the above characteristics tend to occur in patients with severely abnormal EEG background. Further adjustments of seizure detection parameters of the algorithm to detect similar seizures may not add clinical value, and might come at the cost of more false positive detections.

Continuing challenge of artefacts: The artefact detection capabilities of the algorithm have been improved, especially by reducing ECG artefacts. However, as expected when EEG monitoring is done for longer periods in a NICU, more artefacts (cumulative duration *Fp*) were detected, however, the *Fp/h* were improved when compared to the previous version of the algorithm[20]. Both biological and non-biological artefacts offer challenges to automated seizure detection. Technical innovation can help in reducing the false positives due to biological signals like ECG, tremor, respiration etc. The technical details of the improvements made to our algorithm are described in detail elsewhere[21]. External, non-biological artefacts are more challenging and the best solution is for the EEG technician and the clinical neurophysiologist to remain alert to them during the monitoring. Electrode artefacts, pulsation artefacts etc. can be reduced by slightly repositioning the patient's head or the electrodes. In other words, to be successful, EEG monitoring needs to have active interaction between the caregivers, (neurotechnologist, neonatologist, nursing staff and the clinical neurophysiologist). Automated seizure detection is only a tool to be used to reduce the work load and should not be seen as a black box into which EEG data can be fed with a resulting output that gives all the answers for therapeutic decision making. The automatic detections need to be first visually verified and subsequently, the algorithm can help to monitor response to treatment.

The problem of dubious seizures: We encountered two types of dubious seizures. The algorithm performs differently with each type. The first type is the low amplitude arrhythmic discharges with poor repetitiveness seen in four of our patients with very severely abnormal EEG background. These discharges, though occurring paroxysmally, do not fit well into our operational definition of seizures, as their beginning and end are difficult to differentiate from the EEG background and they do not show a clear cut evolution in amplitude and frequency. As expected, the algorithm fails to detect majority of these discharges. Patients showing these patterns had severely abnormal outcome (death or severe disability) despite receiving optimal medical care, including aggressive treatment of subclinical seizure discharges under EEG guidance, comparable to our other patients. We feel that these discharges are signatures of severe underlying cortical injury and treating them aggressively with AED is unlikely to offer a clinical benefit. The

second type of dubious seizure is more difficult to categorize and we encountered these in a patient with a well-defined ischemic stroke involving the right middle cerebral artery territory. They constituted brief rhythmic discharges (BRDs) lasting 5 to7 seconds or periodic lateralized epileptiform discharges (PLEDs). They were localized to the brain regions that had also expressed definite seizures. Though they did not fit well into our definition of seizures (shorter duration, or no clear-cut evolution), many of these discharges were detected by the algorithm. Some authors feel that the BRDs have the same clinical significance as longer electrographic seizures[30]. PLEDs in neonates probably have the same clinical significance as in older children and adults[34]. It is however controversial whether PLEDs constitute ictal or interictal phenomena.

***Clinical significance of seizure detection*:** Automated seizure detection guides treatment decisions, helps prognostication and also helps to study the pathophysiology of seizures in various encephalopathies. From a practical view point, one can argue that this technique is clinically relevant only when the detection of subclinical electrographic seizures results in treatment decisions that ultimately are beneficial to the patient. It would be meaningful only when the brain injury or dysfunction is in a potentially reversible stage. This is best done by combining seizure detection with studying of dynamic changes in EEG background activity[35]. Abnormality of EEG background activity and its evolution over time are ideally suited to assess the severity and course of the underlying encephalopathy[14,27,36]. However, whether the seizure patterns themselves are affected by the severity of hypoxic brain injury, has not been previously studied. With these considerations in mind, we assessed the morphological characteristics of electrographic seizures as well as the performance of the automated seizure detection algorithm in the context of EEG background activity. Even with the relatively small number of patients, we were able to show that seizure characteristics like amplitude, frequency and rhythmicity are influenced by the severity of the underlying encephalopathy and in turn, might affect seizure detection. This type of relevant information is lost when NICUs rely only on simple monitoring tools like aEEG. We are presently using these ictal characteristics in our detection algorithm and these can easily be incorporated into the output/visual display. We feel that the presently achieved

sensitivity and false positive rate is a good balance and that the algorithm is ready for multi-center studies.

More research into the electrographic characteristics of neonatal seizures [37] is needed, which will help us to better understand the phenomena that we are trying to detect. While automated detection algorithm developers are busy with the methodological and technical issues, important research needs to be done in filling the gaps in present knowledge about the pathophysiology of neonatal seizures in various encephalopathies as well as the effect of their treatment.

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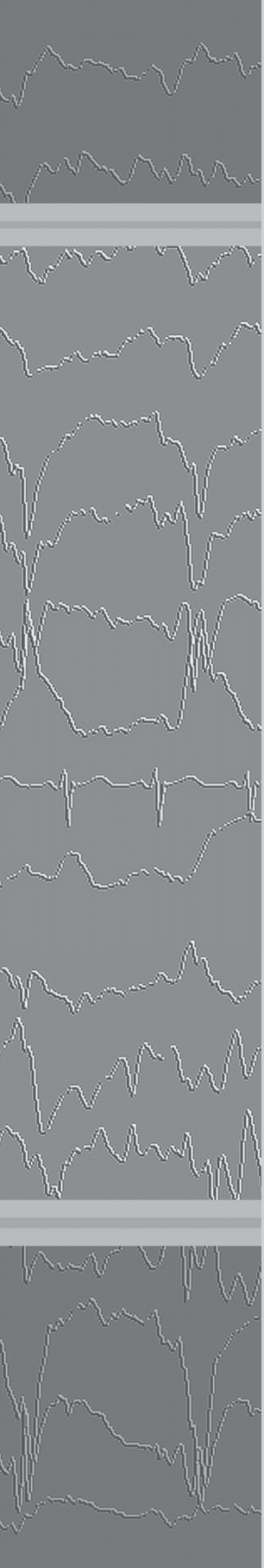


Chapter 4

Discussion & Conclusion

4.1 SUMMARY, DISCUSSION AND FUTURE PERSPECTIVES

Despite rapid advances being made in the care of the sick neonate, the incidence of birth asphyxia (2 to 14/1000 live births) has remained essentially unchanged in most of the developed countries. Reports of reduction in prevalence from the United States and Canada in the last decade is probably an artefact related to the use of stricter International Classification of Diseases codes[1]. Post-asphyxial neonatal seizures occurring in the context of hypoxic ischemic encephalopathy (HIE) continue to be a vexing problem. Majority of these seizures are subclinical, being detected only by continuous EEG monitoring. While there is agreement that these patients tend to have relatively more severe encephalopathy and in turn a poorer outcome, the exact pathophysiological mechanisms underlying post-asphyxial seizures are not well understood. The studies described in this thesis were an attempt to better understand the phenomena of post-asphyxial neonatal seizures.

Electrographic characteristics of neonatal seizures relate to the severity of brain injury (Chapter 2.1)

This study found that EEG characteristics of subclinical neonatal seizures like total seizure number, and seizure burden (cumulative duration of seizures) are strongly related to the severity of abnormalities of EEG background (a sensitive measure of the severity of HIE). We also found that easily recognizable seizure characteristics like spread to contralateral hemisphere, generation of faster (>3Hz) firing frequencies (majority of neonatal seizures show a frequency of 1 to 2 Hz) and increase (>50%) in ictal arrhythmicity were related to increased severity of HIE. Furthermore, this finding was related to the increased occurrence of injury to deep grey nuclei (thalami and basal ganglia, TBG), which is well known to be a strong predictor of poor neuromotor outcome[2]. Similar to a previous study[3], we found that the EEG background abnormality was the strongest predictor of poor outcome and seizure number or burden or presence of TBG injury on MRI did not significantly add to this. Intensive brain monitoring and treatment with antiepileptic drugs (AED) is unlikely to help this group of patients. In patients with moderate encephalopathy, we found a trend towards significance for seizure burden to be associated with poor outcome.

Results similar to ours (increasing seizure burden with more severe brain injury) can be found in previous studies, though this relationship was not highlighted in them. A closer look at a

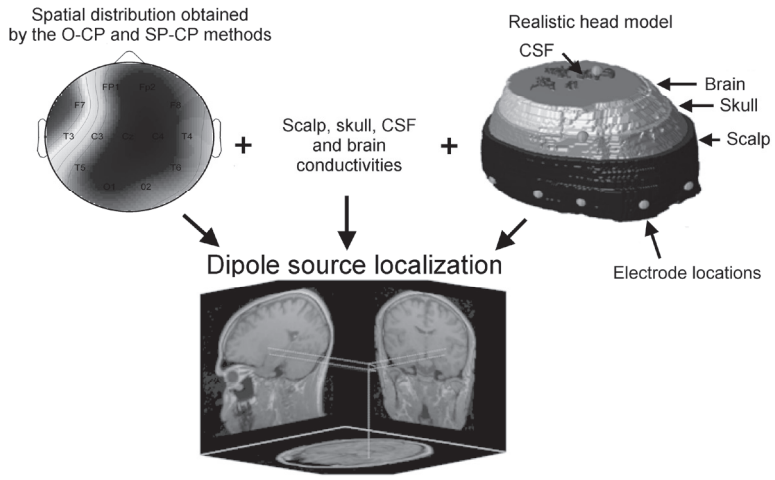
study of prognostic value of continuous EEG monitoring in full term infants with HIE[4] shows that increasing proportion of patients with worsening EEG background activity expressed electrographic seizures. One can also see in the study by McBride et al.[5], (Table 2) that patients with electrographic seizures did indeed have a more severe degree of EEG background abnormality. Also in the recent study by van Rooij et al [6], increasing number of subclinical seizures were recorded by aEEG, in patients with more severe encephalopathy.

Characteristics of seizures relate to patterns of brain injury (Chapter 2.2)

To further explore the relationship of seizures to brain injury, we studied in detail the spatial and morphological characteristics of neonatal seizures and related them to patterns of brain injury seen on MRI. We found clustering of seizure foci in brain regions (central and temporal) known to be selectively vulnerable to hypoxic brain injury in both patients with mild and severe brain injury. Occurrence of more seizures in the frontal regions tended to be related to more severe brain injury. There was also an effect of anatomic location on the firing frequency and rhythmicity of seizures. Though our study suggests that majority of post-asphyxial seizures are associated with cortical injury visible on MRI, there are many unanswered questions. What are the pathophysiological substrates of seizures in patients with relative sparing of cortex? Why do patients with similar appearing patterns of brain injury express seizures variably? Based on the results from this study, we think that neonatal seizure characteristics hold more clinically relevant information (in addition to simple measures like seizure occurrence and seizure burden). Incorporating detailed seizure characteristics to future studies is warranted. Studying the source of neonatal seizures in a realistic head model (Fig 1) as well as quantitative studies of MRI lesion volume may give more information.

Fig 1. *Automated seizure detection and PARAFAC localization[7] combined with source localization in a realistic 3D head model*

(Figure courtesy of W.Deburchgraeve and I. Despotovic)



Heart rate changes during neonatal seizures (Chapter 2.3)

Through this study, we confirmed that seizure related heart rate changes do occur in neonates, even in the presence of severe brain injury, and even when baseline heart rate variability (a normal healthy response of heart rate to autonomic nervous system influences) is lost. Though robust ictal heart rate changes could be detected in only a few patients, we found an interesting relationship of rhythmic fast seizures occurring over the temporal regions with ictal tachycardia. Our results confirm the existence of cerebral hemispheric connections to the brain stem autonomic regulatory centers in the neonate. However, we conclude that heart rate monitoring is not sensitive as a seizure detection tool in perinatal asphyxia.

Development of an automated neonatal seizure detection algorithm (Chapters 3.1 and 3.2)

Continuous EEG monitoring (cEEG) is labor-intensive. The lack of reliable automated methods of EEG analysis and seizure detection is hampering studies of neonatal seizures. With this goal, we set out to develop an automated seizure detection system with the technical collaboration of the department of Electrical Engineering (ESAT) at the Katholieke

Universiteit in Leuven. This system, which mimics a human observer reading EEG, shows a high sensitivity, good positive predictive value and low false positive rates. The algorithm (called *NeoGuard*) was further improved, especially in artefact reduction[8] and then validated in a new, large data set of neonatal seizures. In the second study, we were also able to determine the effects of EEG background abnormality on the performance of the algorithm (EEG characteristics of neonatal seizures were found to vary depending on the EEG background). The overall results indicate that this is a useful tool for long-term EEG monitoring in the NICU. Our aim is to further develop this into a practical tool, widely available to neonatologists for bed-side monitoring of brain function.

Unanswered questions and future perspectives

It is not known why some patients with HIE express seizures while others with similar degree of encephalopathy and structural brain injury as seen on MRI do not. Without properly understanding the pathophysiology, we cannot meaningfully study the effect of neonatal seizures and their treatment. Correlating EEG data with proton magnetic resonance spectroscopy[9] or other methods of molecular imaging, or with biomarkers of brain injury like S100, an astroglial calcium binding protein,[10] may throw more light on the underlying pathophysiological mechanisms. This could help guide therapeutic decision-making as well as help in developing targeted treatments in the future. For instance, our results suggest a trend for frontal seizures to associate with severe brain injury. Aggressive treatment with antiepileptic drugs may not improve the outcome in this group of patients. Same holds true for patients showing a high seizure burden in combination with a severely abnormal EEG background. The seizure characteristics in these patients also appear to be different (increased arrhythmicity, faster frequencies and spread to contralateral hemisphere), suggesting different pathophysiological mechanisms.

Seizure detection: Our algorithm needs further validation on multi-center EEG data. Inter-rater agreement on visual scoring of seizures has to be ascertained on seizure data from different centers, followed by comparison of performance of different seizure detection algorithms on EEGs with varying degrees of background abnormalities. Other algorithms like synchronization likelihood[11] may work better in patients expressing low amplitude arrhythmic oscillations (‘dubious seizures’ as described in our study). The aim of such comparative studies should be to combine the strengths of different algorithms for improved seizure detection.

While developers are busy with the methodological and technical issues of automated EEG analysis, important work needs to be done to fill the gaps in present knowledge about neonatal

seizures. Evidence for using intensive continuous neonatal brain monitoring and treatment of subclinical seizures with AED is incomplete, and much of the present practice is driven by habit or anecdote. Objective evaluation of this labor-intensive and expensive investigation is needed to understand its contribution to improving health. Also needed are large prospective randomized studies to assess the clinical impact of detection and treatment of electrographic neonatal seizures after perinatal asphyxia.

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4.2 SAMENVATTING

Het vóórkomen van perinatale asfyxie in ontwikkelde landen is in de laatste decennia niet veranderd (10 tot 14 per 1000 levendgeboren kinderen). Bij perinatale asfyxie is het optreden van neonatale aanvallen een lastig probleem en een prognostisch ongunstig teken. Aangezien een groot deel van deze aanvallen bij pasgeborenen op de neonatale IC (NICU) subklinisch zijn, kunnen deze aanvallen alleen herkend worden door middel van continue EEG bewaking. De patiënten met subklinische neonatale aanvallen hebben vaak een ernstige onderliggende functiestoornis van de hersenen. De pathofysiologie van neonatale aanvallen na perinatale asfyxie is niet duidelijk en er is geen hard bewijs dat behandeling van deze verschijnselen met antiepileptica de prognose verbetert. De onderzoeken beschreven in het eerste gedeelte van dit proefschrift hadden als doel, het fenomeen van neonatale aanvallen na asfyxie beter in kaart te brengen, en de relatie met onderliggende hersenschade te verduidelijken. Het tweede gedeelte beschrijft een nieuwe methode van automatische detectie van neonatale aanvallen tijdens EEG bewaking van de hersenen.

Electrografische kenmerken van neonatale aanvallen in het EEG zijn gerelateerd aan de ernst van onderliggende cerebrale functiestoornis (hoofdstuk 2.1)

Deze studie heeft aangetoond, dat de electrografische kenmerken van subklinische neonatale aanvallen, zoals totale aantal en duur, sterk gecorreleerd zijn met de ernst van de afwijkingen in het EEG achtergrondpatroon (deze afwijkingen zijn sensitieve indicatoren voor de ernst van de hypoxische encefalopathie of HIE). Wij hebben ook gevonden dat verschillende andere EEG kenmerken van aanvallen zoals een hoge (>3Hz) vuurfrequentie (de meeste neonatale aanvallen hebben een vuurfrequentie van 1 tot 2 Hz), een toename in ictale aritmiciteit (>50%) en spreiding van de aanvallen naar de contralaterale hemisfeer gecorreleerd zijn met de ernst van HIE. Deze bevinding was bij de onderzochte patiënten verder gerelateerd aan de aanwezigheid van laesies van de basale kernen en de thalami (TBG) bij beeldvorming (MRI), welke een bekende predictor zijn voor een ongunstige prognose na perinatale HIE. In deze studie was het EEG achtergrondpatroon de sterkst bepalende factor voor een slechte outcome (gedefinieerd als sterfte of ernstige handicap) en bleek het totale aantal en de duur van de aanvallen geen toegevoegde waarde te hebben voor de predictie van slechte outcome. Bij pasgeborenen met deze prognostisch ongunstige kenmerken (ernstige afwijkingen van het achtergrondpatroon in het EEG en laesies van TBG), draagt intensieve

EEG bewaking en behandeling van subklinische aanvallen waarschijnlijk niet bij aan een betere prognose. Echter, bij patiënten met een matig ernstige encefalopathie was er een trend tot een associatie tussen cumulatieve aanvalsduur en slechte outcome. Behandeling van subklinische aanvallen zou bij deze patiënten mogelijk wel een gunstig effect op de prognose kunnen hebben.

EEG kenmerken van neonatale aanvallen zijn gerelateerd aan de MRI patronen van hersenschade na asfyxie (hoofdstuk 2.2)

Deze studie had als doel om de relatie tussen de electrografische aanvallen en hypoxische hersenschade te verkennen. Neonatale aanvallen bleken het vaakst vóór te komen in de centrale en temporale hersengebieden, zowel bij patiënten met een milde tot matige als een ernstige hersenfunctiestoornis. In de neonatale cerebrale cortex zijn deze gebieden ten opzichte van andere hersengebieden relatief kwetsbaar voor hypoxische schade door verschillen in metabole activiteit, bloedvoorziening en/of exciteerbarheid. Daarnaast was er een trend dat patiënten met een ernstige diffuse functiestoornis meer aanvallen vanuit de frontale gebieden hadden. Er was ook een samenhang tussen de anatomische lokalisatie en de vuurfrequentie en ritmiciteit van de aanvallen. Ritmische snelle aanvallen werden alleen gezien over de temporale gebieden, terwijl trage sinusoidale ritmische aanvallen alleen over de centrale en occipitale gebieden werden gezien. De meeste patiënten met aanvallen lieten op de MRI geassocieerde corticale laesies zien. Echter er bleken ook associaties te bestaan die niet goed te verklaren zijn, zoals het vóórkomen van aanvallen bij pasgeborenen met een relatief gespaarde cortex op de MRI. Een andere onopgeloste vraag is waarom sommige patiënten met vergelijkbare laesies op de MRI een variabele expressie van aanvallen hebben. Gebaseerd op de bevindingen van deze studie, zijn wij van mening dat de electrografische kenmerken van neonatale aanvallen klinisch relevante informatie bevatten naast die van het totale aantal en de cumulatieve duur van de aanvallen. Het belang van deze aanvalskenmerken dient in toekomstige studies onderzocht te worden. Bron-lokalisatie van neonatale aanvallen met gebruik van een realistisch hoofdmodel en quantitatieve studies van het volume van laesies bij MRI kunnen van nut zijn om de relatie tussen de aard en omvang van laesies in hersenstructuren en de kenmerken van neonatale aanvallen te verduidelijken.

Hartritmeveranderingen tijdens neonatale aanvallen (hoofdstuk 2.3)

In deze studie, werd het optreden van hartritmeveranderingen gerelateerd aan subklinische epileptische aanvallen bij pasgeborenen bevestigd. Onze bevindingen gaven aanwijzingen voor het bestaan van functionele verbindingen tussen de cerebrale hemisferen en de autonome centra in de hersenstam op neonatale leeftijd. Ictale hartritmeveranderingen werden ook gevonden bij patiënten met ernstige hersenschade, zelfs bij een interictaal volledig ontbreken van fysiologische variatie in het hartritme. Omdat het optreden van hartritmeveranderingen tijdens neonatale aanvallen slechts in een klein percentage van de neonaten met asfyxie werd gevonden, blijkt bewaking van hartritmeveranderingen niet sensitief genoeg om als een aanvalsdetectie methode bij pasgeborenen met asfyxie toe te passen.

Ontwikkeling van een automatisch neonataal aanvalsdetectie algoritme (hoofdstukken 3.1 en 3.2)

Continue EEG-bewaking op de NICU met behulp van visuele EEG beoordeling is erg arbeidsintensief. Het ontwikkelen van (semi)automatische EEG bewaking met detectie van klinisch relevante veranderingen is voor bredere inzetbaarheid van EEG monitoring van groot belang. Het ontbreken van een betrouwbare automatische aanvalsdetectiemethode is ook een beperkende factor bij wetenschappelijk onderzoek naar onder andere neonatale aanvallen. In nauwe samenwerking met de afdeling Elektrotechniek (ESAT) van de Katholieke Universiteit van Leuven, hebben wij een automatische aanvalsdetectie methode ontwikkeld. Het systeem is gebaseerd op visueel te detecteren kenmerken van neonatale aanvallen in het EEG. De aanvallen worden op basis van morfologie onderverdeeld in ‘reeksen van scherpe golven’, ‘reeksen van ritmische oscillaties’ of een combinatie daarvan. De resultaten tot nu toe zijn veel belovend, met hoge sensitiviteit, goede positief voorspellende waarde en een laag aantal fout positieve detecties. Het algoritme is vervolgens verbeterd door toevoeging van automatische artefactreductie en validatie hiervan werd in een nieuwe grotere data set uitgevoerd (*hoofdstuk 3.2*). In deze tweede studie bleek ook, dat een in toenemende mate afwijkend achtergrondpatroon van het EEG van invloed is op de kenmerken van electrografische aanvallen zoals amplitude, frequentie, duur en ritmiciteit met bovendien een negatief effect op de detectievermogen van het algoritme. De resultaten van de validatiestudies laten zien dat het algoritme (*NeoGuard*) bij de meeste patiënten betrouwbaar genoeg is voor automatische detectie van aanvallen tijdens continue cerebrale monitoring met EEG. Ons doel is om dit verder toe te passen in een ‘bed-side’ monitor van neonatale hersenfunctie.

Suggesties voor verder onderzoek

Het is duidelijk dat subklinische neonatale aanvallen verdere bestudering vergen. Er zijn weinig gepubliceerde gegevens over de klinische relevantie van verschillende electrografische kenmerken van neonatale aanvallen. Grote prospectieve gerandomiseerde studies zijn nodig om de relatieve bijdrage van subklinische (electrografische) aanvallen en de behandeling daarvan met antiepileptica aan de outcome te bepalen. Bij de opzet en analyse van een dergelijke studie is stratificatie van de patiënten gebaseerd op de omvang en de ernst van hypoxische encefalopathie een vereiste. Correlatie van aanvalsdata (inclusief gedetailleerde electrografische kenmerken en bronlocalisatie) met enerzijds het klinische beeld en anderzijds bevindingen bij technieken zoals proton magnetisch resonance spectroscopie, near-infrarood spectroscopie of andere biomarkers van hersenschade, zoals S100 (een astrogliale calcium verbinding eiwit), kunnen meer informatie over de pathofysiologische betekenis van de diverse vormen van subklinische aanvallen opleveren.

Aanvalsdetectie: De validatie van ons aanvalsdetectie algoritme zal uitgevoerd moeten worden in een grotere patiëntenpopulatie in meerdere centra, met aandacht voor het ‘interrater disagreement’ probleem bij het visueel scoren van aanvallen als “gouden standaard”. Daarnaast is het nuttig de resultaten van verschillende aanvalsdetectie algoritmen op dezelfde EEG dataset te vergelijken. Het doel van een dergelijke vergelijkende studie moet zijn om de sterke punten van de detectie methoden te combineren tot een voldoende sensitieve en specifieke, klinisch toepasbare ‘bed-side’ monitor.

APPENDIX

1. EEG background scoring based on evolution of discontinuity & SWC over 24 h

Grade	*DurIBI*	*AmpIBI*	*SWC*	*Improve*	*Reac*	*Variab*	*Other findings*
0	NA	≥25-50	++	NA	+	+	Normal for CA
1	≤5	10-25	+/-	↑SWC	+	+	↑ ShW, <6 sec runs of theta/delta
2	<10	<10	-	↓IBI	+	+	*
3	10-20	<10	-	to grade 2	+	+	-
4	>20	<10	-	to grade 2	+/-	+/-	-
5	10-20	<10	-	No	+/-	+/-	-
6	>20	<10	-	to grade 5	-	-	-
7	10-20	<10	-	Worsen	-	-	↑ IBI >20 sec
8[†]	>20	<10	-	No/worsen	-	-	

SWC: sleep-wake cycles. ***h***: hours, ***CA***: conceptional age. ***NA***: not applicable. ***IBI***: inter-burst interval. ***DurIBI***: length of IBI in seconds. ***AmpIBI***: mean amplitude of IBI in µV. ***Improve***: improvement in 24 h. ***React***: reactivity. ***Variab***: variability. ***ShW***: sharp waves. * Excessive inter-hemispheric asynchrony and amplitude asymmetry were also classified as at least grade **2**. [†] includes also persistent low voltage (<10 µV) and isoelectric EEG background.
↑ increased. ↓ decreased.

2. Classification of post-asphyxial brain injury patterns on MRI [¶]

Pattern	*Description*
1.	Deep grey matter injury with limited cortical involvement
2.	Deep grey matter injury with extensive cortical involvement
3.	Deep grey matter injury with watershed injury
4.	Isolated watershed injury
5.	Isolated white matter injury
6.	Isolated extensive cortical injury
7.	Others

[¶]Swarte R *et al*., Imaging patterns of brain injury in term-birth asphyxia. *Acta Paediatrica* 2009;98:586-92.

LIST OF ABBREVIATIONS

aEEG: amplitude-integrated EEG

AS: active sleep

BS: Burst-suppression

BSID: Bayley Scales of Infant Development

CA: Conceptional age

cEEG: Continuous EEG Monitoring

CP: Cerebral Palsy

DWI: Diffusion Weighted Imaging

ECG (EKG): Electrocardiogram

EEG: Electroencephalogram

EMG: Electromyogram

EOG: Electro-oculogram

GA: Gestational age

GMFCS: Gross Motor Functional Classification System

h: hours

HIE: Hypoxic Ischemic Encephalopathy

Hz: Hertz

IBI: inter-burst interval

MDI: Mental Developmental Index

MRI: Magnetic Resonance Imaging

NICU: Neonatal Intensive Care Unit

PDI: Physical Developmental Index

PP: Post Partum

QS: quiet sleep

sec: seconds

SWC: Sleep-Wake Cycling

TA: *tracé alternant*

TBG: Thalami-Basal ganglia injury on MRI

TD: *tracé discontinu*

WM: White matter injury

WS: Watershed injury

µV: microvolts

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LIST OF PUBLICATIONS

Publications in peer reviewed medical journals

1. Deburchgraeve W, **Cherian PJ**, De Vos M, Swarte RM, Blok JH, Visser GH, Govaert P, Van Huffel S. Time varying neonatal seizure localization. *Methods Inf Med* 2010;49: (Epub ahead of print). DOI: 10.3414/ME09-02-0041.
2. Mathuranath PS, **Cherian PJ**, Mathew R, Kumar S, George A, Alexander A, Ranjith N, Sarma PS. Dementia in Kerala, South India: prevalence and influence of age, education and gender. *Int J Geriatr Psychiatry* 2010; 25: 290-97.
3. Deburchgraeve W, **Cherian PJ**, De Vos M, Swarte R, Blok JH, Visser GH, Govaert P, Van Huffel S. Neonatal seizure localization using PARAFAC decomposition. *Clin Neurophysiol* 2009;120:1787-96.
4. Swarte R, Lequin M, **Cherian P**, Zecic A, van Goudoever J, Govaert P. Imaging patterns of brain injury in term-birth asphyxia. *Acta Paediatr* 2009;98:586-92.
5. **Cherian PJ**, Swarte RM, Visser GH. Technical standards for recording and interpretation of neonatal electroencephalogram in clinical practice. *Ann Indian Acad Neurol* 2009;12:58-70.
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7. van Doorn J, **Cherian PJ**. Breach rhythm related to a solitary skull lesion due to multiple myeloma. *J Neurol Neurosurg Psychiatry* 2008;79:819.
8. Visser AM, **Cherian PJ**, Visser GH. Suppression-burst EEG pattern in a neonate with seizures. *J Clin Neurosci* 2008;15:198, 221.
9. Mathuranath PS, **Cherian JP**, Mathew R, George A, Alexander A, Sarma SP. Mini mental status examination and the Addenbrooke's Cognitive Examination: effect of education and norms for a multicultural population. *Neurol India* 2007;55:106-110.
10. **Cherian PJ**, Blok JH, Swarte RM, Govaert P, Visser GH. Heart rate changes are insensitive for detecting postasphyxial seizures in neonates. *Neurology* 2006;67:2221-23.
11. Mathuranath PS, George A, Mathew R, **Cherian PJ.** Profiles of language impairment in progressive nonfluent aphasia. *Ann Ind Acad Neurol* 2006;9:25-31.
12. Sas AM, **Cherian PJ**, Visser GH. Evolution of stimulus induced rhythmic EEG discharges in three patients with encephalopathy. *Eur J Neurol* 2006;13:908-11.

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14. Mathuranath PS, George A, **Cherian PJ**, Mathew R, Sarma PS. Instrumental activities of daily living scale for dementia screening in elderly people. *Int Psychogeriatr* 2005;17:461-74.
15. Vijai J, Kapoor A, Ravishankar HM, **Cherian PJ**, Kuruttukulam G, Rajendran B, Sridharan R, Rangan G, Girija AS, Jayalakshmi S, Mohandas S, Mani KS, Radhakrishnan K, Anand A. Protective and susceptibility effects of hSKCa3 allelic variants on juvenile myoclonic epilepsy. *J Med Genet* 2005;42:439-42.
16. Mathuranath PS, Hodges JR, Mathew R, **Cherian PJ**, George A, Bak TH Adaptation of the ACE for a Malayalam speaking population in southern India**.** *Int J Geriatr Psychiatry* 2004;19:1188-94.
17. **Cherian PJ,** Radhakrishnan K. Triphasic waves in a patient with brainstem hemorrhage modified by hemispheric infarct. *Eur J Neurol* 2004;11:789-90.
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20. Mathuranath PS, George A, **Cherian PJ**, Alexander A., Sarma SG, Sarma PS. Effects of Age, Education and Gender on Verbal Fluency. *J Clin Exp Neuropsychol* 2003;25:1057-1064.
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22. Vijai J, **Cherian PJ**, Sylaja PN, Anand A, Radhakrishnan K. Clinical characteristics of a South Indian cohort of juvenile myoclonic epilepsy probands. *Seizure* 2003;12:490-496.
23. Jacob A, **Cherian PJ**, Radhakrishnan K, Sarma PS. Emotional facial paresis in temporal lobe epilepsy: its prevalence and lateralising value. *Seizure* 2003;12:60-64.
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26. Pandian JD, Moosa NVA, **Cherian PJ**, Radhakrishnan K. Brainstem abscess complicating tetralogy of Fallot successfully treated with antibiotics alone. *Neurol India* 2000;48:272-275.

27. Radhakrishnan K, **Cherian PJ**. Epidemiology of epilepsy. *NIMHANS Journal* 1999;17:389-399.

Chapters in books

1. **Cherian PJ**, Radhakrishnan K. Epilepsy in developing countries: diagnostic evaluation. In, Murthy JMK, Senanayake N, eds, *Epilepsy in the tropics*. chapter 7. Landes Bioscience 2006, Austin, Texas. ISBN: 978-1-57059-692-6.

2. **Cherian PJ**, Venugopal A, Radhakrishnan K. Video EEG monitoring- an assessment of its clinical utility and efficacy. In, Radhakrishnan K ed, *Medically refractory epilepsy* p 113-118, published by Sree Chitra Tirunal Institute for Medical Sciences and Technology, 1999 Thiruvananthapuram. ISBN: 81-901111-0-8.

3. **Cherian PJ**, Alexander A, Radhakrishnan K. The role of Wada's test in the presurgical evaluation of patients for temporal lobectomy. In, Radhakrishnan K ed, *Medically refractory epilepsy* p137-143, published by Sree Chitra Tirunal Institute for Medical Sciences and Technology, 1999 Thiruvananthapuram. ISBN: 81-901111-0-8.

ABOUT THE AUTHOR

Joseph Cherian Perumpillichira was born in Trichur in India, on 13th March 1967. After completing his secondary schooling at St.Paul's High School and Farook College, he joined the Government Medical College, Calicut, in 1984 for medical studies. After obtaining his MBBS degree in 1991, he did his residency in Internal Medicine (Department heads, Prof. D.K. Mitra and Prof. P.D. Mitra) at the B.J. Medical College in Pune, and upon completion in 1994, obtained his M.D. in Internal Medicine. He then worked for 6 months as a lecturer in Internal Medicine (Prof. K.A. Salim) at the government medical college, Calicut. From 1995, Joseph underwent the three-year Neurology residency program at the Sri Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST) at Thiruvananthapuram (Prof.K. Radhakrishnan) and upon its completion, obtained the D.M. degree in Neurology.

In 1998, he worked for 10 months as a neurologist at Lisie Hospital, a mission hospital in Cochin, before joining back SCTIMST as assistant professor in Neurology. Here he developed his interest in Clinical Neurophysiology, especially in long-term EEG monitoring (non-invasive and invasive) of patients with refractory epilepsy. He was actively involved in the care of patients in the comprehensive epilepsy program as well as the neurobehavioral clinic. His research at SCTIMST involved clinical as well as epidemiological studies of epilepsy and cognitive disorders.

In 2003, he joined as a neurologist/clinical neurophysiologist (staff member), the section of Clinical Neurophysiology in the department of Neurology (Prof.dr. P. Sillevis Smitt) at Erasmus MC, University Medical Center at Rotterdam, in the Netherlands. Here, in addition to patient care and teaching of Neurology residents, he has been actively involved with the EEG monitoring program in the neonatal ICU, and the development of an automated neonatal seizure detection method in collaboration with the department of Electrical Engineering at the Katholieke Universiteit, Leuven, Belgium. Joseph lives in Bergschenhoek, near Rotterdam, with his wife Linu and sons Arvind and Athul.

ERASMUS UNIVERSITEIT ROTTERDAM

PhD Portfolio

Summary of PhD training and teaching

Name PhD student: Joseph Cherian Perumpillichira Erasmus MC Department: Neurology, Section of Clinical Neurophysiology	PhD period:2003-2010 Promotor: Prof.dr. W.F.M. Arts Supervisor: Dr.G.H.Visser	
1. PhD training		
	Year	**ECTS**
General courses		
- Statistics course: Erasmus winter program, Rotterdam, NL	2008	2
- Introductory Biostatistics for researchers, University of Utrecht, NL	2008	4
Presentations		
- Long-term EEG monitoring in neonates after perinatal asphyxia. NVLKNF meeting, Utrecht, NL	2003	0.5
- Polygraphic registrations in the NICU. NVLKNF meeting, Ede, NL	2005	0.5
- Oral presentation of research work at quarterly meetings with universities of Leuven and Gent	2007-2010	4
Presentations at International conferences		
- Ictal heart rate changes in neonatal seizures. Child Neurology Society, Ottawa, Canada (poster presentation)	2004	1
- Electroencephalographic characteristics of neonatal post hypoxic seizures. Asian & Oceanian symposium in Clinical Neurophysiology, Chiang Mai, Thailand (poster presentation)	2005	1
- The accuracy of aEEG in detecting post-asphyxial neonatal seizures. Child Neurology Society, Pittsburgh, USA (oral presentation)	2006	1
- Posy-asphyxial facio-mandibular tremor in neonates: a poor prognostic sign. European Pediatric Neurology Society, Kusadasi, Turkey (poster presentation)	2007	1
- Comparison of C3-C4 versus P3-P4 aEEG channels for seizure detection and 6 versus 30cm/h time bases for EEG background classification in neonates with perinatal asphyxia. American Epilepsy Society, Philadelphia, USA (oral presentation)	2007	1
- EEG and clinical characteristics of post-asphyxial neonatal seizures. Brain Monitoring and Neuroprotection in the Newborn, Vienna, Austria (poster presentation)	2008	1
- Post-asphyxial neonatal seizures: clinical, EEG and radiological features. NOS-M symposium on early brain development, Helsinki, Finland (oral presentation)	2009	2

Other meetings attended		
- Neonatal brain injury and neuromonitoring. Goteborg, Sweden	2003	0.5
- Brain monitoring and neuroprotection in the newborn. Florida, USA	2009	0.5
2. Teaching activities		
	Year	**ECTS**
Patient demonstration and teaching		
- Bi-weekly neonatal EEG monitoring presentations for neonatologists, neurology residents and neurotechnologists (oral presentation)	2004-2010	2
Supervising practicals, Tutoring		
- Teaching Neurology residents about neonatal EEG monitoring in the ICU and supervising patient care	2004-2010	8
Total		30