Background aEEG/EEG measures in very preterm infants

Relation to physiology and outcome

SVERRE WIKSTRÖM
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Abstract

The overall aim of this thesis was to characterize single-channel aEEG/EEG, recorded during the first postnatal days in preterm infants, in relation to brain function and two-year outcome.

Study I investigated if aEEG/EEG was associated with neonatal brain injury, inflammation and outcome in 16 very preterm (VPT) infants. The interburst interval (IBI) was prolonged, and aEEG amplitudes were lower in infants with brain injury, and in infants developing handicap. Cord blood TNF-α correlated with IBI.

Study II investigated inter-rater agreement of visual burst detection, as compared to automated burst detection based on a non-linear energy operator (NLEO) in an EEG data set from 12 extremely preterm (EPT) and 6 VPT infants. The sensitivity of the NLEO was 64 % and 69 % (EPT and VPT infants, respectively) and the specificity 96 % and 88 %. The algorithm was then modified to further improve the accuracy.

Study III investigated if arterial carbon dioxide and plasma glucose is associated with EEG continuity. In 247 sets of samples (PaCO₂, plasma glucose, IBI) from 32 EPT infants there was a positive association between PaCO₂ and IBI; higher PaCO₂ was associated with longer IBI. Corrected for carbon dioxide, plasma glucose had a U-shaped association with IBI in infants with good outcome.

Study IV investigated the predictive value of aEEG/EEG in 41 EPT and 8 VPT infants. All VPT infants had good outcome. Predictors of outcome in EPT infants included presence or absence of burst-suppression, continuous activity and cyclicity, median IBI and interburst%. Seizures were associated with neonatal brain damage but not with outcome.

Improved preterm brain monitoring may in the future be used for early identification of infants at high risk of brain damage and adverse outcome, which may have implications for direction of care and for early intervention.

Keywords: Electroencephalography, brain damage, prediction, interburst interval, Neurodevelopmental impairment

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Dedicated to the memory of my mentor

Conny Nordin,
Professor of General Psychiatry and Royal Court Chaplain.
Thank You for inspiring me to clinical research, for all the bad jokes and for challenging discussions on ethics.

“Seek and You will find” (Matthew 7:7)
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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<td>aEEG</td>
<td>Amplitude integrated EEG</td>
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<td>aEEG/EEG</td>
<td>aEEG with corresponding EEG</td>
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<td>ASA</td>
<td>Acute stage abnormalities</td>
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<td>BS</td>
<td>Burst-suppression</td>
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<td>BSID-II</td>
<td>Bayley scales of infant development II</td>
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<td>CBF</td>
<td>Cerebral blood flow</td>
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<td>CPAP</td>
<td>Continuous positive airway pressure</td>
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<td>cUS</td>
<td>Cerebral ultrasonography</td>
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<td>DC</td>
<td>Discontinuous</td>
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<td>EEG</td>
<td>Electroencephalogram</td>
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<td>EPT</td>
<td>Extremely preterm</td>
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<td>FFT</td>
<td>Fast Fourier transformation</td>
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<td>GA</td>
<td>Gestational age</td>
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<td>IBI</td>
<td>Interburst interval</td>
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<td>IQR</td>
<td>Interquartile range</td>
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<td>IVH</td>
<td>Intraventricular haemorrhage</td>
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<tr>
<td>ln</td>
<td>Natural logarithm</td>
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<tr>
<td>MDI</td>
<td>Mental developmental index</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>NDI</td>
<td>Neurodevelopmental impairment</td>
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<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<td>NLEO</td>
<td>Non linear energy operator</td>
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<tr>
<td>NPV</td>
<td>Negative predictive value</td>
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<tr>
<td>PaCO₂</td>
<td>Partial pressure of carbon dioxide in arterial blood</td>
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<tr>
<td>PDI</td>
<td>Psychomotor developmental index</td>
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<tr>
<td>PNA</td>
<td>Postnatal age</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value</td>
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<tr>
<td>PVL</td>
<td>Periventricular leucomalacia</td>
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<tr>
<td>SAT</td>
<td>Spontaneous activity transient</td>
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<td>SWC</td>
<td>Sleep-wake cycling</td>
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<td>TD</td>
<td>Tracé discontinu</td>
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<td>VPT</td>
<td>Very preterm</td>
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<td>WMD</td>
<td>White matter damage</td>
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Introduction

This thesis work, which was performed with the overall aim to contribute to improved care of vulnerable preterm infants, is based on studies investigating features of single-channel EEG background in infants born at 22-30 weeks gestational age (GA) in relation to perinatal factors and outcome.

Preterm birth

According to the World Health Organization (WHO), preterm birth is defined as birth at less than 37 completed gestational weeks. Infants who participated in the studies in this thesis were born ‘very preterm’, before 32 gestational weeks or ‘extremely preterm’, i.e. before 28 completed gestational weeks. Gestational age was, in almost all infants, estimated from fetal ultrasound at 17-18 gestational weeks which is standard in Sweden.

WHO has estimated that preterm birth constitute 10% of all births worldwide. Preterm birth is accordingly an important cause of neonatal morbidity in the world [1]. In Sweden 4.8% of all births were registered as preterm during 2008 [2] and 1% of the children are born very preterm, i.e. around 1000 infants in Sweden annually. About 20-30% of preterm births are attributed to iatrogenic preterm delivery, i.e. due to maternal or fetal conditions such as preeclampsia, intrauterine growth restriction. Spontaneous delivery after preterm labour occurs in around 70-80% of preterm births. The cause is not always identified (idiopathic preterm birth) but especially in very early preterm labour and preterm premature rupture of the membranes, concurrent intrauterine infection and inflammation is common [1, 3]. Statistical risk factors linked to preterm birth include medical conditions of the mother or fetus, socioeconomic factors, genetic influence and infertility treatments [1].

Survival and outcome of very preterm infants

During the 1980’s and 1990’s survival of extremely preterm infants (EPT) increased as a result of improvements in antenatal and neonatal care. In a large study cohort of extremely preterm infants, the EPICure study, including infants born in the UK and in Ireland during 1995, survival rates to dis-
charge of live born infants were: 11% at 23 weeks GA; 26% at 24 weeks and 44% at 25 weeks [4]. EXPRESS (EXtremely PREterm infants Study in Sweden), a more recent Swedish population based observational study of infants born at 22-26 weeks GA between 2004 and 2007, reported a 1-year survival in live born infants of 10% at 22 weeks GA; 53% at 23 weeks; 67% at 24 weeks, 82% at 25 weeks and 85% at 26 weeks GA [5].

The increased survival in EPT infants is associated with higher rates of ‘intact survivors’ and lowered prevalence of cerebral palsy [6-9], but also with an increase in the absolute number of surviving infants with neurodevelopmental impairment (NDI) [6]. Attention is increasingly focused on the long-term neurocognitive morbidities in the extremely preterm survivors [10]. In the EPICure cohort 24% of the children had severe disabilities at 30 months [11], and when tested at 11 years age 45% of the children had serious functional disability compared with 1% of control classmates. Cerebral palsy occurred in 17%, but the most prevalent impairment was suboptimal cognitive function (in 40%) [10]. Reduced cognitive performance in extremely preterm born individuals seems to persist into late adolescence [12-13] and EPT born children are at increased risk for later psychiatric disorders, including attention-deficit-hyperactivity disorder (ADHD), mood disorders, and autism spectrum disorders [13-15].

"Illness severity indices” or ”risk indices” are commonly used for assessing mortality or morbidity risks, especially for adjustments of study populations in quality of care assessment and clinical research. Such scores include various items of physiological risk factors that can be recorded during early postnatal life. Both the revised clinical risk index for babies (CRIB-II) and simplified version of score for neonatal acute physiology (SNAPPE-II) have primarily been validated as predictors of mortality risks, but also evaluated for predictors of NDI [16-18].

**Brain damage in preterm infants**

Brain injury associated with haemorrhagic lesions and white matter damage (WMD) is common in very preterm infants. Periventricular and intraventricular haemorrhages (IVH) appear on neonatal cerebral ultrasonography (cUS) in 10-45% of EPT infants, most often during the first three postnatal days [5, 19-21]. The incidence of IVH increases with decreasing GA [5]. The “intraventricular haemorrhage” (IVH) originates in the highly vascular periventricular germinal matrix. The severity of IVH is usually classified according to Papile [22] (Figure 1): Grade I: a subependymal germinal matrix haemorrhage; Grade II: intraventricular progress of a germinal matrix bleeding without dilatation of the ventricle system; Grade III: intraventricu-
lar haemorrhage with ventricular dilatation; and Grade IV: refers to a haemorrhagic periventricular infarction.

The long-term consequences of IVH’s are variable. Children with IVH grade I or II have, in some studies, no increased risks of handicap. However, EPT infants seem to have an additional risk of cerebral palsy in association with minor IVH due to their immaturity [19, 23]. Intraventricular haemorrhage grade III is overall associated with a 50% risk of neurodevelopmental handicap, and in IVH grade IV the risk is about 80%.

The risk of IVH is closely related to GA, but also to individual intrinsic fragility of the germinal matrix vasculature and to fluctuations in cerebral blood flow (CBF) [20, 24]. Fluctuations in CBF may be related to hypotension and hypertension, and loss of autoregulation, (‘pressure passive regulation’) which is common in unstable extremely preterm babies [25-26]. Furthermore, hypercapnia during the first postnatal days is associated with an increased risk for severe IVH in preterm infants [27].

White matter damage (WMD) may be focal or diffuse (Figure 1), and the aetiology is multi-factorial. Cystic periventricular leucomalacia (cPVL), or focal WMD is associated with ischaemia and is nowadays quite rare; on histological evaluation it is recognized as necrosis and macroscopic cyst formation. Diffuse WMD is an ischaemic and/or inflammatory chronic disturbance involving myelination of the white matter, and is characterized by injury to the myelin producing oligodendrocytes, astrogliosis and infiltration of microglia [28]. Diffuse WMD is a common finding on magnetic resonance imaging (MRI) studies of extremely preterm infants [29]. The border zones between long penetrating arteries and the end zones of short penetrating arteries (‘watershed areas’), where the circulation of the immature brain is not yet fully developed, are the most vulnerable regions for WMD [30]. Preterm infants with moderate-severe WMD on MRI at term equivalent age have a very high risk (50-70%) for developing later NDI [29].

Perinatal infection and fetal inflammatory response including the cytokines TNF-α, IL1-β and IL6 may initiate preterm labour and is also strongly associated with increased risk for WMD in preterm infants [31-32]. Cytokines act locally and have short half life after secretion [33]. In the brain they can be produced by microglia and astrocytes and especially interleukin-1 (IL-1) and Tumor Necrosis Factor-alfa (TNF-α) contribute directly to cytotoxic injury to immature oligodendrocytes and neurons [30].
Cerebral lesions can be diagnosed by cerebral ultrasonography (cUS) and MRI (and by autopsy in non-survivors). Repeated cUS examination is the standard bedside method for early neuroimaging of preterm infants in the NICU. Diffuse echodensity is often the first sign of WMD. Periventricular echodensities persisting for more than seven days are usually considered to be evidence of established WMD. Even though cUS is less reliable than MRI for detection of diffuse or subtle white matter damage [34-35], major lesions detected by cUS are associated with lower IQ and impairment at school age [35]. Among EPT infants without neonatal signs of major structural pathology on repeated cUS, neurodevelopmental impairment may still occur in as many as 30% of the infants [19, 36]. Optimal investigations for detection of WMD, as well as gray matter injury, are preferably performed at term equivalent age by using MRI [29].

The electroencephalogram (EEG)

The electroencephalogram (EEG) is a recording over time of brain derived voltage gradients from electrodes placed over the scalp. The sources of the EEG signal are synchronized postsynaptic currents evoked mainly in the vertically oriented cortical pyramidal cells [37].

Figure 1. Illustration of IVH grades I, II-III and IV respectively, cPVL and diffuse WMD. Figure by Siw Wikström
The EEG activity is modulated by interaction between cortical and subcortical (e.g. thalamus) structures and thereby synchronized, which results in rhythms of the cortical activity. During the second trimester a structure called the subplate zone is present in the fetal brain. The subplate is the origin of thalamo-cortical and cortico-cortical afferents that build up during development and is critical for modulation of preterm EEG activity [38].

The EEG, including continuous long-term monitoring, provides an approach to assessment of brain function and dysfunction in real time [39-40], and will thereby also provide additional information to structural cerebral imaging.

The normal EEG of the preterm infant

For clinical purposes conventional neonatal EEG recordings are generally performed with 8-20 electrodes positioned according to the International 10-20 System modified for neonates [41].

Interpretation of neonatal EEG demands knowledge of normal EEG development from early preterm to post term age. In general, neonatal EEG activity has been discussed in terms of the development from a discontinuous to a continuous EEG [42-43]. The normal EEG background of extremely preterms, called *tracé discontinu* (TD) [43-44], shows a basically dichotomous pattern. Periods of low voltage activity, called interburst intervals (IBI), alternate with high voltage ‘bursts’ of activity with mixed frequency content. Several normal transients, e.g. frontal sharp transients and temporal theta (-bursts), have been identified in the preterm EEG, and can be useful for maturational assessment of the EEG [43, 45].

The frequency distribution of the discontinuous preterm EEG, is dominated by delta activity, with as much as 80% of the relative power within frequencies below 1 Hz in infants <32 weeks GA [46] while theta and alpha activity is increasingly recognized towards full term. A correlation between postconceptional age and power of the delta and theta bands, respectively, has been reported. [46]

In the bursts of normal preterm EEG, fast activity is superimposed on slow waves of high amplitude [45]. Such complexes have also been named ‘spontaneous activity transients’ (SATs) [47-48]. This spontaneous and intermittent activity has in animal models been recognized as a specific phenomenon that occurs during brain development [47, 49-51] and is suggested to be instrumental for wiring neuronal networks [47, 49, 51].
It is important to recognize that ‘burst’, when mentioned in the context of bursts in the discontinuous preterm EEG, denote ‘physiological activity bursts’ or ‘SATs’. In contrast, ‘bursts’ as in ‘burst-suppression’ specify a different entity, a pathological EEG pattern with iso-electric IBI [43, 52] that can be seen in patients of all ages after major cerebral insults or during heavy sedation.

With increasing maturation, the duration of bursts increase and the IBIs become shorter, and there is also gradually more ongoing EEG activity during the IBIs [42-43, 45, 48]. In this way EEG background activity changes gradually towards higher continuity [43, 48]. It has been postulated that the gradual development of continuous activity is physiologically distinct from the development of bursts, and that it demands functional thalamocortical and cortico-cortical connections. These develop during the third trimester and consequently this activity is usually not appearing in EPT infants [47].

Several studies have provided reference data for IBIs at different maturational levels, based on visual quantification of preterm conventional EEG [42, 53-59]. Different definitions of bursts and IBI have been used, for example numerical criteria on maximum signal voltage during IBI varied between 15 µV or 30 µV (Hahn, Vecchierini and Biagioni, Selton, Hayakawa, Victor respectively). Hahn et al. consequently demonstrated a considerable difference in IBI values depending on which definition (i.e. thresholds) that was used [55]. Strict amplitude criteria are limited in that they are specific to the recording device, filter settings and electrode distances. Still, a summary of reference data show that in all studies the maximum IBI was below 60 seconds, and mean IBI was 6-14 seconds, in healthy infants at 25-28 weeks GA [42, 53, 55-56, 58].

Changes in behavioural state, observed as alterations in eye movement, respiration, heart rate, overall movements and muscle tone, are associated with alterations in EEG activity. At term age quiet sleep (QS) is characterized by high-voltage slow activity or discontinuous EEG (‘tracé alternant’) [43-44]. Active sleep is characterized by continuous EEG (and rapid eye movements, irregular breathing and some body movements) and wakefulness by continuous EEG (and by general body movements). Arousal and sleep states are regulated by brain stem and hypothalamic connections of the ascending reticular activating system [60]. In this manner sleep-wake cycling (SWC) as expressed in the EEG reflects specific physiological correlates of functional connectivity.

Cyclic variation of the EEG background has been reported in infants from 25 gestational weeks, although more commonly appearing after 27-30 weeks [61-64]. It remains somewhat unclear whether very early appearing of
cyclicity in the EEG background is equivalent to later appearing sleep stages. It was previously validated in amplitude-integrated EEG (aEEG) that cyclicity at 32-34 gestational weeks corresponded to QS and active sleep/wakefulness, respectively [65].

**Abnormal preterm EEG**

Abnormal features in the preterm EEG can be characterized as acute or chronic stage abnormalities. This categorization of the EEG has proved to be very useful since acute changes in the electrocortical activity recover in a similar way, also after severe insults. Persisting chronic stage abnormalities are commonly markers of established injury [66].

Acute stage abnormalities (ASA) appear during and after an insult, and include changes in continuity (longer IBI and absence of continuous patterns), frequency (attenuation of faster frequencies) and amplitude (lowered amplitude range) with associated loss of SWC and reactivity [66]. Presence of such EEG features has been associated with haemorrhagic and ischaemic brain damage in preterm infants [67-70]. Maruyama et al showed, in very preterm infants, that the maximum degree of ASA during the first postnatal days was associated with the risk for later development of cerebral palsy (CP) and also correlated with the severity of the CP [71].

Chronic stage abnormalities denote persisting and characteristic EEG abnormalities appearing when ASA have disappeared. These include dysmature and disorganized patterns and have been associated with poor neurodevelopmental outcome in very and moderately preterm infants [66, 72-74]. A dysmature pattern is characterized by a maturational delay of more than two weeks. Increased IBI alone is difficult to assign to either acute or chronic abnormalities as it may represent either an acute suppression or an immature pattern. Disorganized patterns are characterized by abnormal background patterns and waveforms, e.g. positive rolandic sharp waves (PRSW), which are markers of established WMD and predictive of cerebral palsy if frequent [74].

**Single-channel aEEG/EEG**

Single- or double-channel aEEG/EEG have become readily available techniques for continuous non-invasive monitoring of brain function in both infants and adults [40, 75-76].
The amplitude integrated EEG (aEEG) trend

The aEEG is a trend measure and a monitoring technique, derived from a single bipolar EEG montage. It has its’ origin in the Cerebral Function Monitor (CFM) designed in the 1960’s for continuous monitoring of resuscitated adults [77]. A single- or two-channel EEG is recorded from a pair of bi-parietal electrodes or from two pairs of electrodes, one pair over each hemisphere (commonly frontal-parietal or frontal-central leads) [39]. The EEG processing to aEEG includes asymmetric band pass filtering which attenuates activity below 2 Hz and above 15 Hz in order to reduce artefacts and technical interference. Other important steps of aEEG processing is rectifying and smoothing of the signal, time compression and especially a semi logarithmic display (linear at 0-10 µV and logarithmic display above this voltage) in order to increase sensitivity for detection of changes in low amplitude background activity [39, 78]. In this way the aEEG technique enables continuous assessment of long term changes in cortical background activity. Modern aEEG trend recording is always combined with display of the original EEG signal from the same recording channels. This facilitates discrimination of true EEG findings from artefacts. The term ‘aEEG/EEG’ in this thesis denotes a single-channel EEG recording from which the aEEG trend or other measures may be derived, together with a possibility to assess the corresponding original EEG trace, while ‘aEEG’ indicates that only the aEEG trend was assessed or possible to assess.

The aEEG trend is, like the conventional EEG, mainly interpreted through visual pattern recognition, including assessment of continuity and discontinuity, appearance of sleep-wake cycling and identification of seizure activity. The aEEG’s sensitivity to changes in the low voltage range also makes this trend prone to external interference which often elevates the lower border of the amplitude span when the electrocortical activity is depressed. Consequently, quantitative analysis of aEEG maximum and minimum amplitudes should be performed with caution. In the discontinuous preterm EEG, the lower border of the aEEG trend does normally define the peak-to-peak amplitude of the IBI, but may also be influenced by the duration of the IBIs [39]. The upper border on the other hand, represents the peak-to-peak amplitude of bursts. In some recent monitoring devices these amplitudes can also be directly quantified from the filtered signal. Artefacts, mainly from muscle activity and electrical interference, may disturb long term aEEG monitoring and reduce its’ clinical value [79-80], which is especially important to consider in the extremely preterm population, potentially vulnerable even to non-invasive EEG monitoring.

The very early aEEG background pattern has a very high predictive value in asphyxiated term infants [81]. Although the clinical value of aEEG and
aEEG/EEG is less established in preterm infants, several authors have reported reference values in stable preterm infants (Table 1 a and b). In parallel with EEG development, also aEEG background becomes increasingly continuous with maturation, and the most stable maturational feature seems to be increasing amplitude of the lower border during the most discontinuous part of the recording (i.e. QS periods in more mature preterm and term infants), if cycling is present. Furthermore, some investigators have also reported correlation between early aEEG abnormalities and preterm brain damage [61, 82-85]. Only a few studies have reported the prognostic value of early aEEG/EEG in VPT and EPT infants [61, 84-86], and to our knowledge only two previous studies reported outcomes beyond the neonatal period. There is, consequently, a need for more studies assessing the relation between long-term monitored aEEG/EEG background features, neonatal morbidity and long-term outcome. Furthermore, to aid the visual analyses there is also a need for studies applying quantitative measures reflecting the preterm discontinuous EEG.

Table 1 a and b. (Following pages). Summary of studies on aEEG and aEEG/EEG in stable preterm infants. LB = Lower border of the aEEG trend; UB = Upper border of the aEEG trend; DC = Discontinuous; PNA = Postnatal Age; PMA = Postmenstrual Age.
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<td>Viniker et al [87]</td>
<td>PCA 30-43 w, n=107, Normal at discharge</td>
<td>UB and LB aEEG amplitudes</td>
<td>Rising LB = strongest maturational feature</td>
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<td>Thornberg, Thiringer [88]</td>
<td>Term, preterm (n=10 and 19) Normal at 18 months</td>
<td>Serial aEEG (n=56); UB and LB amplitude, SWC</td>
<td>Increasing GA associated with gradual increase of LB; Average duration of quiet sleep 19-20 minutes</td>
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<td>Kuhle et al [63]</td>
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<td>Sleep wake cycling (SWC)</td>
<td>SWC appeared at mean GA 28 weeks and median PNA 6 days</td>
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<tr>
<td>Burdjolov et al [89]</td>
<td>GA 24-39 w, n=30, No HIE, IVH or PVL, no sedative, no malformation,</td>
<td>Serial aEEGs. Scores for continuity, cyclicity, LB amplitude, bandwidth and sum score.</td>
<td>Sub scores and total sum score correlated with GA and PCA; Cyclicity, continuity and bandwidth was the most sensitive indicators of maturation</td>
</tr>
<tr>
<td>Olischar et al [64]</td>
<td>GA 23-29 w, n=75, Stable, normal ultrasound and neonatal outcome</td>
<td>aEEGs first 2 weeks; Pattern: discontinuous high/low voltage, continuous, bursts/h;</td>
<td>Higher GA associated with more continuous activity and fewer bursts/hour;</td>
</tr>
<tr>
<td>West et al [90]</td>
<td>GA 2431 w, n=63; Neonatal survival, no IVH2-4 or PVL</td>
<td>aEEG daily first week; Automated quantification: Activity above different amplitude thresholds; Median aEEG</td>
<td>Continuity and median amplitude increased for every day over the first week Trend for decrease in SEF during the first week</td>
</tr>
<tr>
<td>Sisman et al [91]</td>
<td>GA 25-32 w, n=31, No seizures, IVH 3-4, PVL</td>
<td>Serial aEEGs (n=119); Pattern: Continuity; SWC, Low/high base voltage, Upper high voltage, Span</td>
<td>aEEG matured with PMA; Development of continuity accelerated in EPT infants.</td>
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<tr>
<td>Author</td>
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<tr>
<td>Klebermass et al [92]</td>
<td>GA 23-29 w, n=98, No ventilation, sepsis or hypotension, no IVH/PVL,</td>
<td>Relative duration of aEEG patterns: discontinuous high/low voltage, continuous; SWC ≥20 minutes</td>
<td>Higher GA and higher PNA associated with increased likelihood of continuous activity and lower likelihood of DC low voltage pattern</td>
</tr>
<tr>
<td>Herbertz et al [93]</td>
<td>GA 24-34 w, n=56, No IVH III-IV, normal neonatal outcome material.</td>
<td>Repeated aEEGs (n=92); Pattern: continuous; discontinuous high/low voltage; nearly isoelectric</td>
<td>Higher GA and higher PNA associated with more continuous activity</td>
</tr>
<tr>
<td>Kuint et al [94]</td>
<td>GA 24-35 w, n=32, No IVH/PVL, sedation, or malformation; Normal neonatal outcome</td>
<td>Serial aEEGs (n=86); Cycle characteristics in developed SWC.</td>
<td>Increasing maturation associated with decreased intercycle bandwidth and increased intercycle LB; No cycles at 24-30 w PCA</td>
</tr>
<tr>
<td>Soubasi et al [95]</td>
<td>GA 25-34 w (PMA 25-42 w), n=96, Stable, no ultrasound abnormality; Normal neonatal outcome</td>
<td>Serial aEEGs first 72 h then weekly to discharge (n=624); Pattern: discontinuous high/low voltage, continuous; SWC; Bandwidth</td>
<td>Increasing GA/PMA was associated with increased continuity and SWC, more mature bandwidth; At the same PNA: lower GA and higher PNA associated with more mature aEEG</td>
</tr>
<tr>
<td>Niemarkt et al [96]</td>
<td>GA &lt;32 w, n=18, Neonatally stable AGA, No: asphyxia, IVH&gt;1, sedative, or BPD; Normal at 1 y.</td>
<td>Weekly aEEGs (n=79; 4-6/infant); Automated quantification of aEEG amplitudes and % of activity with lower margin amplitude &lt; 5 µV</td>
<td>First week: GA correlated with aEEG lower margin amplitude and correlated negatively with % activity &lt; 5 µV. GA and PNA contributed equally to lower margin amplitude and % of activity &lt; 5 µV</td>
</tr>
<tr>
<td>Niemarkt et al [97]</td>
<td>GA &lt;32 w, n=18, Neonatally stable AGA, No: asphyxia, IVH&gt;1, sedative, or BPD Normal at 1 y.</td>
<td>Weekly single-channel EEGs from first week (4-6/infant); Automated quantification of IBIs by NLEO-based detection algorithm.</td>
<td>Increasing PMA associated with decreased IBI,</td>
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Monitoring EEG continuity measures

Previously used methods for visual quantification of aEEG or long-term recorded limited-channel EEG include burst counts (aEEG), measurement of IBI (conventional EEG), and continuity assessments of the aEEG/EEG background, e.g. to measure the percentage of recording with amplitudes of the aEEG or original EEG above a certain voltage threshold IBI [53, 84-86, 90]. Just like the case of aEEG amplitudes, however, reliance on strict amplitude measures may be sensitive to interference “adding to” the EEG signal amplitude.

Based on physiological considerations [47-48], detection and long-term monitoring of bursts/SATs and IBIs in the preterm could provide useful information for evaluation of functional maturation and overall brain activity [47].

Algorithms have been developed for detection of EEG bursts and interburst intervals utilising various signal features [98-100]. A segmentation algorithm by Särkelä et al [98] was developed for adult EEG, and software based on this algorithm is currently available in the Nervus/NicoletOne system (Care-Fusion, San Diego CA, USA). The basic idea of the segmentation algorithm is to use the output of a non-linear energy operator (NLEO), as presented by Plotkin and Swamy [101], which classifies electrocortical activity as either ‘burst’ or ‘interburst’. NLEO values reflect both amplitude and frequency content of the analysed signal. Absolute values of NLEO values are smoothed by a sliding window. If the smoothed NLEO value exceeds a burst threshold during a defined period of time, EEG is classified as burst. If the same value stays below another threshold for a certain period of time, then EEG is classified as ‘suppression’ (i.e. interburst, in a normal discontinuous preterm EEG). If neither of these conditions is met, or if the NLEO value exceeds an artefact threshold, the EEG is classified as artefact/continuous EEG. See Figure 2 for an example of NLEO-based segmentation.
Factors affecting preterm EEG and brain function

Cerebral haemorrhagic lesions

Development of an IVH is associated with acute EEG and aEEG depression, the degree and duration of the EEG depression associated with the severity of the IVH [67-69, 82, 84, 102]. It is also previously shown in children surviving with large IVH’s, that the number of bursts/hour in the aEEG trend during the first 48 hours is predictive of outcome [84]. Suspected seizures, and even status epilepticus, were frequently seen in association with IVHs in early aEEG recordings, however, without possibility of verification by the original EEG [61, 82]. Seizures were also detected in conjunction with echodense lesions (haemorrhages and/or WMD) in two-channel EEG recordings [103]. Absence of sleep wake cycling is usually associated with the overall depression of electrocortical activity in conjunction with development of haemorrhagic lesions.

White matter damage

Hypoxic-ischaemic insults, including low cerebral blood flow associated with hypcarbia, may be associated with acute changes in the EEG. However, the correlation between appearance of WMD and a previous possible insult is often difficult to establish. As a consequence, acute and early EEG changes during development of WMD are difficult to assess with certainty.
due to the difficulty in establishing an early reliable diagnosis of WMD. Chronic changes appearing in the EEG, such as positive rolandic sharp waves (PRSW) are indicators of established brain injury, and especially presence of PRSW is regarded as a marker of white matter injury and predictive of an increased risk for cerebral palsy [74]. Disorganized and dysmature EEG patterns [70, 73] have also been identified as sensitive markers of WMD. A correlation between severity of WMD and decreased EEG Spectral Edge Frequency (SEF) was also found in one study, with some indication that EEG changes may be present early in WMD development [104]. In a recent study, the severity of both acute- and chronic stage abnormalities were correlated with severity of PVL, with the most important association during the first days and during the second week, respectively [70].

**Cerebral ischaemia**

Low cerebral blood flow (CBF) has been coupled to transient EEG or aEEG background depression in preterm infants [105]. In preterm infants, low cardiac output and hypotension have been associated with EEG suppression [106-107]. At low mean arterial blood pressure (<23 mm Hg) relative delta power decreased, EEG amplitudes were lowered and IBIs were prolonged [107]. Gavilanes et al demonstrated very clearly in newborn piglets, how aEEG amplitudes decreased in conjunction with acute anemic hypotension and returned when blood pressure and blood volume were restored [108].

**Carbon dioxide**

Carbon dioxide has a depressant effect on central nervous system activity, in its extreme form known as carbon dioxide narcosis. Three previous studies indicate that high arterial carbon dioxide levels in preterm infants are associated with EEG background depression and changes in brain stem auditory responses [109-111]. The mechanisms for these reactions are not known in detail, although effects on ion-channels via intra- or extracellular cerebral pH have been suggested. A reversible association between increasing carbon dioxide levels and EEG, mainly slowing and amplitude depression, is well known from adult subjects and animal studies [112].

Carbon dioxide is also a powerful regulator of cerebral blood flow (CBF) [25, 113]. Hypocarbia may through vasoconstriction impair CBF and a clinical relevant association between early hypocapnia and ischaemic brain injury is established [114-115]. Hypercarbia on the other hand, cause vasodilatation and has been associated with increased risk of hemorrhagic damage [27]. Experimental data from neonatal rats also indicate that moderate hypercarbia could be neuroprotective, while extreme hypercarbia has been linked to increased risk for brain damage [116-117].
Glucose

Both hypoglycaemia and hyperglycaemia are common in extremely preterm infants due to immature physiology and need for parenteral nutrition [118]. Although hypoglycaemia was previously considered to be the main risk, hyperglycaemia is currently increasingly recognized as a risk factor for brain damage and death in very preterm infants [119-120]. Experimental data also indicate that hyperglycaemia makes the brain more vulnerable to ischaemic insults [121-122], and this may consequently be a contributing factor to brain injury in unstable preterm babies.

Only a few studies have addressed neurophysiological effects from hypoglycaemia in newborns [123-125]. Koh et al showed that sensory evoked potentials were attenuated at blood glucose levels lower than 2.6 mmol/L in children (including five newborns). This level has since then been regarded as the lowest accepted level for neonatal blood glucose in many neonatal intensive care units, since it was also supported by epidemiological data [126].

Medications

Administration of certain medications such as sedatives, opioids, antiepileptics and surfactant commonly depress electrocortical activity to an extent that the evaluation of EEG or aEEG is affected. A bolus dose of morphine can suppress an otherwise normal neonatal EEG, and especially in preterm infants turn a normal discontinuous pattern into burst-suppression [127]. Not only bolus doses, but also infusion of morphine may cause profound suppression of EEG background [128].

The effect on the EEG is dependent on immaturity, dosage and timing of administration in relation to the recording [127, 129-131]. However, little is known about the duration of specific drug effects on the EEG, but recent studies indicated considerable inter-individual variation in recovery time after bolus doses of antiepileptics [132]. A study assessing effects of the ‘InSureE’ procedure (‘intubation, surfactant, extubation’) on aEEG in preterm infants showed prolonged depression of the background activity, up to 24 hours after administration of 100 µg/kg morphine [133]. It is also shown that caffeine increase aEEG amplitudes throughout a 2-hour period after administration of a bolus dose [134].

Perinatal inflammation

Beside the important role in the pathogenesis of WMD, TNF-α also modifies synaptic activity and may cause cell membrane hyperpolarisation [135], making it still more interesting in terms of acute neural dysfunction in rela-
tion to inflammatory response. One study has previously investigated possible associations between perinatal inflammation, early EEG and brain injury in preterm infants [136]. However, that study could not demonstrate any association between endotoxin levels after birth, acute stage abnormalities in the EEG, or development of PVL, respectively. In contrast, data from fetal sheep suggested that changes in very slow EEG activity could be used to detect effects of inflammation on cerebral brain function [137]. Furthermore, a recent publication stated that EEG delta activity increased in association with chorioamnionitis in fetal sheep, and also reported a correlation between delta activity and cortical, as well as white matter, microglia activation [138].
Aims

The overall aim of this thesis was to characterize single-channel EEG and aEEG of very preterm infants in relation to specific influences on brain function by using long-term monitoring techniques with focus on quantitative analysis of EEG background. The specific aims are listed below.

Study I
To investigate if quantitative measures of the early single-channel aEEG/EEG are associated with neonatal brain injury, perinatal inflammation and long-term outcome in very preterm infants.

Study II
To test the inter-rater reliability of visual burst detection in the early preterm EEG and to assess visual burst detection in the early preterm EEG in relation to an automated burst detector based on a non-linear energy operator (NLEO).

Study III
To investigate if arterial carbon dioxide and plasma glucose levels are associated with EEG continuity and frequency content during the first days after birth in extremely preterm infants.

Study IV
To describe early aEEG/EEG of very preterm infants in relation to two-year outcome and to identify aEEG/EEG features predictive of outcome. Furthermore to compare the prognostic information of single channel EEG monitored over several hours versus time restricted recording quantified with an automated algorithm for interburst interval detection.
Patients and Methods

Ethical considerations

The clinical studies were performed at Lund University Hospital and study protocols approved by the Regional Ethical Review Board at Lund University.

Any physical risks from EEG monitoring are considered to be very low and to our knowledge only minor skin irritation after adhesive tapes has been reported by others, although not to our awareness in the current studies. Very thin subdermal needle electrodes were used in study I. This type of electrode has been part of standard monitoring of infants, before the introduction of hydrogel electrodes, and they seem to cause very little discomfort. We have not recognized any infections or other side-effects of these electrodes. The hydrogel electrodes, used in studies II-IV, are gentle to the very sensitive skin of extremely preterms after soft preparation of the skin [39]. Still there are, of course, ethical issues that need to be addressed when setting up studies involving newborn infants. The main ethical issue second to risk is the patients’ autonomy. The infants have not themselves decided on their participation in a study. In the present studies informed and written consent was obtained from the parents, in line with the Helsinki Declaration [139]. In the discussion of consent, an important aspect is the assumed position of dependence towards the clinicians, experienced by parents of extremely preterm infants during the first postnatal days of their children. In this case, consent was performed prior to delivery, before the initiation of NICU care.

The studies are descriptive and will not benefit the participating individuals. The Helsinki Declaration states that incompetent subjects (e.g. preterm infants), ”must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden”. We propose that the potential gain of knowledge regarding preterm neurophysiology, with the purpose of future improvements in neonatal care, meets this criterion.
Clinical settings in the NICU

The infants in the included studies were all born at Lund University Hospital, Sweden, a regional referral perinatal center with a tertiary level neonatal intensive care unit (NICU). Gestational age was in all cases determined by prenatal ultrasound, usually performed at 17-18 weeks of gestation according to Swedish routines. All included infants received antenatal steroids. According to Scandinavian tradition, early treatment with continuous positive airway pressure (CPAP) was encouraged and extremely preterm infants were not routinely intubated after birth. Nevertheless, a majority of infants still needed early surfactant and were supported by mechanical ventilation. Moderate permissive hypercapnia with a PaCO$_2$ goal of 5 to 7 kPa was practiced during mechanical ventilation. Peripheral oxygen saturation was aimed at a range of 88 to 92% in extremely preterm infants, and mean arterial blood pressure at, or above, a level corresponding to the infant’s gestational age in weeks. Umbilical venous and arterial catheters as well as peripheral arterial catheters were inserted on clinical indication. Arterial blood gases and plasma glucose values were checked repeatedly as clinically indicated, and analysed in the NICU within a few minutes after sampling.

Most extremely preterm infants received oral feedings with donor breast milk from the first hours from birth and were also supported with i.v. 10% glucose infusion, with additional amino acid and lipid infusions after the first 24 hours [140]. An overall aim was to keep plasma glucose levels between 3 and 6 mmol/L. When study I was performed, the clinical standard of practice was to administrate continuous infusion of morphine (10-20 (µg/kg)/h) to mechanically ventilated infants. In the other studies, bolus doses of morphine (0.05-0.1 mg/kg), and sometimes continuous morphine infusion, were administered as indicated by validated pain scores to mechanically ventilated infants, but not routinely.

Diagnosis of neonatal brain injury

Infants in the reported studies had repeated cUS performed on days 1, 3 and 7, at 3 and 6 weeks, and at term equivalent age. The examinations were performed by experienced neonatologists, and image data were also reviewed by paediatric radiologists. Germinal matrix and intraventricular haemorrhages were classified according to Papile [22]. White matter damage was defined as periventricular echodensities persisting more than seven days, or periventricular cysts [141]. Cerebral MRI at term equivalent age was performed in infants participating in studies III and IV but data were not included in the present studies.
EEG recordings

A single-channel EEG was the basis for analysis in all four studies. The EEG was recorded in the NICU at Lund University Hospital with a Nervus/NicoletOne EEG System with U16 amplifier, sampling rate 256 Hz, high pass filter at 0.16Hz and low pass filter at 500 Hz (Taugagreining HF, Reykjavik, Iceland/CareFusion, San Diego CA, USA). Electrodes were applied at the P3 and P4 positions according to the International 10-20 System, plus a frontal reference electrode. In study I thin subdermal needle electrodes were used and in studies II, III and IV hydrogel electrodes (Ambu® Neuroline, Ambu A/S, Ballerup, Denmark). All recordings were made during the first three days from birth, as soon as possible after initial stabilization at the NICU.

In studies I, III and IV, the aEEG trend was used at 6 cm/h for an initial identification of major artefacts, ongoing interference (e.g. from high frequency oscillatory ventilation) and seizure activity. Artefacts, interference and seizures activity thereafter confirmed in the single-channel original EEG were excluded from analysis. After selection of appropriate parts of the recordings (see below) quantitative aEEG and EEG output were exported to text files that were used for calculations.

aEEG trend analysis

In study IV, the aEEG trend, displayed at 6 cm/h, was visually rated in 4-hour blocks by two investigators together, blinded for the patients’ identity and clinical data. The assessment of the aEEG background pattern was repeatedly aided by inspection of original single-channel EEG at 15 mm/s and 100 µV/cm sensitivity. The aEEG background pattern of every epoch was interpreted as burst-suppression, discontinuous or continuous according to Hellstrom-Westas et al, [142] and thereafter the activity dominating the 4-h epoch was further classified into 6 categories: 0 = Inactive/flat, 1 = Sparse BS, 2 = Dense BS, 3 = Mix of both BS and discontinuous (DC), 4 = Discontinuous (DC), 5 = Continuous (C). Cyclicity was rated in 4 categories: 0 = Absent, 1 = Imminent, 2 = Established but immature, 3 = Developed and mature.

In study I, minimum and maximum amplitudes of the aEEG [39] were identified in 1-second epochs with the Nervus/NicoletOne software (exported to text files) and averaged during the whole study period.
Single-channel EEG analysis

For visual detections of bursts in paper II, we selected an 11-minute epoch from each recording from places where the traces contained minimal artefacts and consisted of clear epochs of *tracé discontinu*. Three investigators (SW, LHW, SV) chose visual criteria (see below) for burst identification and each reader marked the duration of all bursts in the selected EEG epochs. Raters used the same display settings: filtering 0.16-30 Hz, sensitivity 200 µV/cm, time base 15 mm/s. A burst was defined as a distinct occurrence of cerebral activity with a slow (< 2Hz) component and associated faster activity. Burst onset was defined as a clear deviation from the relatively inactive background activity (i.e. interburst). Due to the filter-generated rebounds of the slow components in the preterm EEG [143], the end of the burst was defined by the visual appearance of the trace returning to baseline (i.e. IBI), rather than a strict return to zero line.

In study IV, seizure activity was identified in the original single-channel EEG by an experienced neurophysiologist (IR) who examined all recordings (in total 2614 hours) at 10 mm/s and 70 µV/cm sensitivity. The duration of each seizure was measured and it was also retrospectively determined whether the seizure was detectable in the aEEG trend at 6 cm/h. Seizure activity was characterized by: A sudden appearance of an abnormal electrical event; lasting 10 seconds or more; with evolving, repetitive waveforms that gradually built up and then declined in frequency, morphology, or amplitude [144].

Bursts and IBI were detected using the segmentation algorithm implemented in the Nervus/NicOne software. In study I the interburst intervals were averaged over the whole study period 0-72 hours postnatal age. In study III, interburst intervals were averaged from 10-minute artefact free epochs of EEG during the interval between 15 to 5 minutes before each blood sample analysis. In study IV, median interburst intervals were calculated 1-hourly over the whole study period and over four time intervals (0-24 h, 24-48 h, 48-72 h and 0-72 h). Interburst% (percentage of recording detected as interburst, analogue to burst% in study II) was calculated over the same epochs.

In study I, the median non-filtered peak-to-peak amplitude in single-channel EEG was calculated for a comparison with the aEEG amplitude measures.

Power spectral analysis in study III was performed using Fast Fourier Transformation (FFT) with a time base of 10 seconds. Spectral power was analysed from artefact-free 5-minute epochs, obtained between 10 and 5 minutes before sample analyses. For every 5-minute epoch, total and relative
(%) band power was calculated within the following frequency bands: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-30 Hz).

For comparison of findings in long term continuous monitoring and an intermittent time restricted recording, one hour of high quality single-channel EEG was selected from each of the original long term recordings in study IV. This data set was selected as closely before 48 hours age as possible and met the following criteria: No ongoing artefacts or technical interference as identified in P3-P4 original EEG at 70 µV/cm, no handling of the infant according to charts or obvious from EEG artefacts and no morphine administered during the previous three hours. If periodic cycling activity was visually present in aEEG the 1 hour epoch was selected 30 minutes before to 30 minutes after maximum continuity according to the aEEG trend. For the selected one hour epochs, the same quantitative measures of EEG as above were calculated.

Follow up

Surviving children had neurodevelopmental follow up at two years corrected age. A psychologist evaluated the children with the Bayley Scales of Infant Development (BSID-II) [145], including two subscales, Mental scale and Motor scale. Scores were transformed into Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI), both with mean indices of 100 and SD of 15. Children with MDI or PDI scores below 50 were assigned a score of 50.

The infants were also examined by an experienced clinician, who was unaware of the EEG findings, performing a standardized neurological evaluation, including the Neurologic Optimality Score [146]. The Neurologic Optimality Score has a maximum score of 78 and numbers below 74 are considered suboptimal.

In study I, the overall neurodevelopmental function was also classified according to Scheffzek, a composite outcome for classification of handicap level that can be scored from the neurological and cognitive tests [84, 147]. The Scheffzek classification includes five categories: the lowest category, 0, denotes a neurodevelopmentally healthy child while a severely multihandicapped child will be classified in category 4. In study IV, neurodevelopmental impairment (NDI) was defined as presence of cerebral palsy, or MDI <70, or PDI <70, or blindness, or deafness. Good outcome was defined as surviving without NDI, and poor outcome was defined as death or surviving with NDI at 2 years corrected age.
Patients and methods in study I

Patients

Sixteen preterm infants (GA median 25.5 w, range 24-28) were prospectively recruited between October 2001 and February 2003. All infants received surfactant and required mechanical ventilation during the first postnatal days.

Nine infants had neonatally diagnosed brain damage; five developed IVH grade 1-3, three had IVH grade 2-3 with WMD, and one infant had isolated WMD. In the other seven infants no abnormalities were detected by cUS. All 16 infants survived and had neurodevelopmental follow-up at two years of corrected age.

Methods

Single-channel aEEG/EEG-monitoring was initiated at a median (range) postnatal age of 36.2 h (11.5-69) and with duration 18.6 h (3.0-55.3). The duration of the analysed EEG from each infant was 16.6 h (3.0-50.3).

Blood samples were taken from the umbilical cord at birth and from arterial catheters at 6, 24 and 72 hours postnatal age. Nine cytokines (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-α and IFN-γ) were analysed in plasma using cytometric bead array and flow cytometry technique as previously described [148].
Material and methods in study II

Dataset

The dataset used in this methodological study consisted of 10-minute segments of single-channel EEG tracings from 12 extremely preterm (EPT) and 6 very preterm (VPT) neonates randomly chosen from the cohort of 54 infants (without knowledge about clinical data) described in study IV. Gestational ages among the EPT neonates were 23-27 weeks, and in the VPT group 28-30 weeks.

Methods

Three independent raters marked the recordings for activity that was classified as ‘bursts’, without knowledge about clinical data. Visual detections of the raters were compared at each sampled time point (256 points per second). The proportion of overall agreement was evaluated in order to obtain a single value describing the inter-rater agreement in each recording. Three descriptive parameters of bursts were derived for each recording from the markings of each rater: number of bursts, average burst duration and proportion of time covered by bursts (burst%). Additionally, the frequency spectrum of epochs that were unanimously detected as either burst or interburst was calculated using Fast Fourier Transformation (FFT).

Two different versions of the NLEO algorithm for automated segmentation were implemented. The first version was constructed in MatLab to mimic the algorithm used in the NicOne software for burst detection (already described). In the second version modifications were done (see paper II) in order to further improve the accuracy of detection. Results of the automated detections were compared with a gold standard in which only epochs where all three raters agreed on the classification of the EEG (burst/interburst) were included (Figure 3).

Figure 3. Study work flow in paper II. With permission from the Publisher
Patients and methods in study III

Patients

Thirty-two extremely preterm infants with median (range) GA 25 w (22-27) were included between July 2005 and May 2007. The infants constitute a sub-group of the infants in study IV. Included infants had at least three paired samples including arterial blood gas/plasma glucose and good quality EEG preceding the 15 minutes before the blood gas analysis, with at least 2 hours since morphine administration. Out of the 45 extremely preterm infants described in study IV, 13 were excluded from this analysis due to lack of such data.

Twenty-seven of the 32 infants needed mechanical ventilation, and five were supported by CPAP. Twenty-nine of the infants survived the first week and in 26 of them there were no signs of a large IVH (defined as grade III-IV) according to cUS. Furthermore, no infants had WMD diagnosed with cUS.

Methods

In total 247 sets of samples were included in the analysis, each sample consisting of a measure of PaCO₂, plasma glucose, IBI and EEG relative and total band power (n=185 for EEG power samples). The median (range) number of included samples per infant was 8 (3-17).
Patients and methods in study IV

Patients
Fifty-four infants were prospectively included between July 2005 and May 2007, 45 of the infants were born extremely preterm (EPT) at less than 28 weeks’ GA, and 9 were born very preterm (VPT) at 28-30 weeks’ GA. During the first 72 hours from birth, infants were cared for according to clinical routines, except for recording of a single-channel EEG, and additional blood sampling of cytokines and growth factors [149].

Methods
Single-channel EEG recording was initiated at a median postnatal age of 8 hours with median (range) duration 56 (14-71) hours per infant. Single-channel aEEG/EEG analysis was performed as already described. The Clinical Risk Index for Babies (CRIB-II) [16] was calculated from data obtained within the first 6 hours. Surviving infants had follow up at two years corrected age (Bayley II and neurological examination).
Statistical analysis

For group comparisons in study I the two tailed Mann-Whitney U-test was used. Spearman rank correlation coefficient was used for correlations.

In study II the three raters proportion of overall agreement was calculated according to Fleiss [150]. Use of kappa statistics was avoided because it is not suited for dependent samples like EEG time series [151]. Confusion matrices were calculated at ‘point-by-point’ level for detection algorithms versus golden standard (i.e. points when all three raters agreed). Based on these, accuracy, sensitivity and specificity of the algorithms were evaluated.

In study III, data were analysed as cross-sectional time-series together with multivariate regression splines [152]. Regression splines are functions that do not require a particular model assumption (linear, quadratic, cubic) for the dependent variable. Instead a set of functions (spline bases) are chosen during estimation from a general, larger set of functions. IBI and EEG power variables were used as outcome variables in regression analyses and were transformed using the natural logarithm (ln) to obtain approximately normal distributions. PaCO₂, plasma glucose, postnatal age and gestational age were entered as predictor variables in a backwards stepwise procedure. For comparison with previously published data [111], we analysed a restricted data set, comprised of measurements at one particular point of time in every infant, namely the first collected sample after 24 hours age. This dataset of strictly independent observations was analysed by linear regression with multivariate regression splines. Plasma glucose levels were also divided in four categories, representing strategies of the clinical care. In order to test for differences in EEG variables between glucose categories we used panel-data regression with glucose category as a single predictor variable.

In study IV, non-parametric statistics were chosen for group comparisons and for comparisons of EEG measures between separate postnatal intervals. In the extremely preterm group IBI, interburst% and aEEG amplitudes of the respective intervals (also including the low artefact 1-hour recordings), CRIB-II scores and ultrasonographic findings were one at a time evaluated as predictors of outcome in logistic regression models. ROC-curves for prediction of outcome were created and optimal cut-off values were determined from the values of combined sensitivity and specificity (likelihood ratio). Sensitivity, specificity, and positive and negative predictive values (PPV, NPV) for prediction of poor outcomes were calculated for aEEG/EEG features, CRIB-II scores and cerebral ultrasonography.
Results

Results in study I

Clinical data are presented in Table 2. The IBI, averaged over the study period, wereas prolonged in the nine infants with neonatal brain injury (IVH of any degree and/or WMD) as compared to the seven infants without injury, median (range) 11.8 (9.6-23.2) sec versus 8.2 (7.1-11.6) sec (p = 0.005). Both the maximum and minimum aEEG amplitudes were lower in infants with neonatally diagnosed brain injury, median (range) 22.5 (12.5-30.9) µV versus 31 (20.7-43.5) µV (p = 0.013) and 11.1 (6.1-14.6) µV versus 15 (10.2-20) µV (p = 0.009), respectively.

Table 2. Neonatal data for infants in study I. Values are medians (ranges). * p<0.05 difference between infants with and without handicap at 2-years corrected age.

<table>
<thead>
<tr>
<th></th>
<th>No handicap (Scheffzek cat. = 0)</th>
<th>Handicap (Scheffzek cat. = 1-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=8</td>
<td>n=8</td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>27 (24-28)</td>
<td>25 (24-28)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>955 (660-1205)</td>
<td>881 (680-1205)</td>
</tr>
<tr>
<td>Apgar score, 5 min</td>
<td>7 (3-9)</td>
<td>6 (5-8)</td>
</tr>
<tr>
<td>IVH/WMD (any grade) (n)</td>
<td>2*</td>
<td>7</td>
</tr>
<tr>
<td>Caesarean section (n)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Antenatal steroids (n)</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

The eight infants with normal neurodevelopmental outcome had significantly shorter IBIs than infants with handicaps, median (range) 8.7 (8.1-23.2) seconds vs 11.8 (9.6-14.1) seconds, respectively (p=0.036). The maximum and minimum aEEG amplitudes also differed according to Scheffzek category, Neurological Optimality Score and PDI. There were no associations between peak-to-peak EEG amplitude and brain injury or neurodevelopmental outcome.

Cord blood TNF-α showed a correlation with IBI (r_s = 0.595; p = 0.025). We recognized no other direct correlations between cytokine levels and aEEG/EEG measures.
Results in study II

Overall agreement of burst detections, at point-by-point level, between raters was 86% and 81% (EPT and VPT neonates respectively). See Figure 4 for an example of visual detection. Burst% showed a significant pair wise correlation between all raters and in both groups of infants. In manual markings, the burst duration increased with increasing maturation: Median burst duration by the three raters were 4.3, 3.1 and 2.9 s, respectively, in extremely preterm infants, and 4.4, 4.8 and 3.3 s, respectively, in very preterm babies. Although very few bursts were longer than 10 s, burst lengths were variable between 1-10 s with a non-Gaussian positively skewed distribution. There was a peak in the middle frequency range (4-8 Hz) in bursts only.

The time points classified the same way by the NLEO algorithm and in agreed visual assessment were 87% and 79% of all sampled time points (EPT and VPT infants, respectively). The sensitivity of the original algorithm was 64% and 69% (EPT and VPT infants, respectively) and the average specificity was 96% and 88%.

Figure 4. Detection of bursts in a VPT infant. Red bars indicate detections in the P3-P4 derivation by three raters. Unanimous detections in pink (‘burst’) and blue (“interburst”). Courtesy of Sampsa Vanhatalo.
Results in study III

There was a positive association between PaCO₂ and IBI; higher PaCO₂ was associated with longer IBI. This association was only present in samples from the 26 infants with good outcome. A 1 kPa increase in PaCO₂ was overall associated with a 9% increase in IBI but the association between PaCO₂ and IBI was stronger in the subset of data restricted to the first sample after 24 postnatal hours, a 1 kPa increase in PaCO₂ was there associated with a 16% increase in IBI.

There were significant non-linear associations between PaCO₂ and EEG power in all frequency bands, but only in the 157 samples from infants with good outcome. The association was strongest in the delta band. Total EEG power reached a maximum at a PaCO₂ of 5.1 kPa and was attenuated both at higher and lower PaCO₂. There were no associations between PaCO₂ and relative band power.

Corrected for carbon dioxide effects, plasma glucose had a U-shaped association with IBI in infants with good outcome. The lowest IBI appeared at a plasma glucose level of 4 mmol/L. There were no correlations between plasma glucose and EEG band power measures. Figure 5 shows the association between categorized plasma glucose and IBI.

![Figure 5](image-url)

**Figure 5.** Box plot demonstrating interburst intervals in relation to plasma glucose, but only from samples where simultaneous PaCO₂ was within the intended range (5-7 kPa).
Results in study IV

Twenty-two EPT and all eight VPT infants survived without NDI at two years of age (good outcome) while 8 EPT infants died in the neonatal period and 11 survived with NDI, i.e. 19 EPT infants had poor outcome.

Figure 6. Background (BG) score development during 0-72 postnatal hours. Background activity increased significantly from 0-24 hours to 24-48 hours but only in infants with good outcome.

Figure 6 shows the trends in background pattern over the study period. Some degree of cyclicity was identified in 25 out of 41 EPT infants, more often in infants with good rather than poor outcome (p = 0.029).

Seizures were identified in the single-channel EEG from 21 infants (43%). In total 89 seizures, duration 12 seconds to 6 hours 10 minutes (median 60 s) were identified. Seizures were associated with neonatal brain damage but not with outcome.

Significant predictors of outcome in EPT infants included: presence or absence of BS (aEEG background score ≤2) (p<0.001), continuous activity (aEEG background score = 5) (p = 0.038) and EEG cyclicity (p = 0.029).

The highest combined sensitivity and specificity for prediction of poor outcome was obtained from interburst% in the 1-hour low artefact recordings with a cut-off interburst% value >50% (Figures 7 and 8). A 10%-units increase in interburst% was associated with an odds ratio 2.0 for poor outcome (p = 0.005).

Findings of IVH or PVL during the first three days were not overall predictive of outcome. CRIB-II scores were lower in EPT infants with good versus poor outcome (p = 0.036). When studying survivors only, there were differences in both visual and quantitative EEG background measures but not regarding CRIB-II scores, depending on outcome (NDI) at follow up.
Figure 7. Interburst% during the 1 hour low artefact EEG recordings close to 48 hours age. Data shown for EPT infants with good and poor outcome (n = 22 and n = 18 respectively), and VPT infants (n = 7), all with good outcome.

Figure 8. Interburst% in 1-hour low artefact EEG recordings, close to 48 hours age. Data shown for EPT survivors with and without NDI (i.e. presence of cerebral palsy, or MDI <70, or PDI <70, or blindness, or deafness) at two years corrected age.
Discussion

The results of the present thesis, obtained from analysis of early single-channel aEEG/EEG recorded over many hours, demonstrate that the electrocortical background activity is an early marker of brain damage in very preterm infants. In line with results in previous studies, the present data support the use of single-channel aEEG/EEG in preterm infants for monitoring aiming at early identification of brain damage. The early aEEG/EEG background is also associated with other factors, including perinatal inflammation since increased levels of TNF-α in cord blood were associated with aEEG/EEG depression. No previous study has reported such an association between pro-inflammation at birth and EEG activity. It was also demonstrated that PaCO₂ and glucose levels considered to be within physiological ranges were associated with the most continuous EEG background in extremely preterm infants. Study III also showed that increased EEG discontinuity may occur at carbon dioxide levels that are used in many NICUs for mechanical ventilation as part of lung protection strategies.

Study IV demonstrates that although the accuracy of prediction is moderate, early aEEG/EEG may be a better predictor of long-term outcome than cranial ultrasound abnormalities during the first week, or a clinical risk index such as the CRIB-II. The predictive value of 1-hour low artefact recordings at 48 hours age was at least equal to that of long term monitoring. This finding points out the importance of high recording quality and absence of drug induced EEG suppression during recording. Furthermore, we also demonstrate that brief epileptic seizures are common in extremely preterm infants during the first postnatal days, and that their presence is not invariably associated with a poor prognosis.

Besides common pattern recognition of aEEG background, automated detection of bursts and interburst intervals was performed in these studies. Study II includes a detailed technical analysis of this method and also shows that inter-rater reliability is confidently high in visual detection of bursts in the preterm EEG. We further conclude that the evaluated NLEO-based segmentation algorithm is capable of accurate detection of bursts in early preterm EEG and that it therefore is valid as a scientific tool in clinical studies.
Visual vs. Automated analysis of EEG background

All aEEG/EEG assessments begin with a visual inspection of the recording, and include an evaluation of the quality of the recording and presence of possible artefacts. This evaluation is the beginning of a structured evaluation of the aEEG/EEG trace, which is essential both for clinical monitoring and for research.

The aEEG/EEG recordings can be assessed according to several scoring systems, but in the current studies we have classified aEEG background patterns corresponding to established EEG terminology in order to be able to investigate and compare physiological findings. Furthermore, we repeatedly inspected the original single-channel EEG trace in order to confirm that the aEEG pattern was consistent with the original EEG background, since artefacts such as high-frequency ventilation may severely influence the appearance of the aEEG trend. For seizure assessment, we analysed the original single-channel EEG trace since previous studies have shown that brief seizures will not appear in the aEEG trend.

As expected, most very preterm infants had discontinuous background patterns during the first postnatal days, although more continuous activity was seen in a few infants and associated with good long-term outcome. We also showed that BS was associated with poor outcome. However, this basis of evaluation probably works best in a one-way direction: the absence of BS supports a favourable prognosis in the same way as ‘better than expected’ presence of continuous background pattern. Although pattern recognition is an accepted method that has proven to be useful both for the clinical situation and for research, additional quantitative measures (e.g. burst or interburst measures) may provide a more precise identification of brain damage and prediction of neurodevelopmental outcome. This is likely to be especially important for analysis of long term recordings where detailed inspection is time consuming and investigation of specific transients and symmetry is limited or lost due to the single-channel mode of recording.

The results of the presented studies support that automated quantitative analyses of EEG continuity (e.g. burst or interburst measures) are valuable in single-channel EEG monitoring, and that the analyses are in line with visual assessment by pattern recognition of the aEEG trend. Study I was, to our knowledge, the first to utilise automated analysis of IBIs over prolonged periods of time. Previous studies have manually quantified IBIs in very preterm infants after visual analysis of the EEG recordings [42, 53, 55-59]. A disadvantage with manual estimation of IBI is that it is extremely cumbersome, and consequently this has previously not been done in long-term recordings. However, the present detector algorithm also has limitations when
data are collected over several hours, since it requires visual exclusion of EEG epochs with major technical interference.

The NLEO-based burst detector that was used in these studies already performs relatively well in the youngest age groups. However, as the neonatal EEG matures to a less dichotomous form [43-45] bursts become less prominent. Consequently, it can be expected that the distinction between bursts and interbursts is progressively more difficult with increasing GA, and our results may be applicable to earliest preterm ages only. The method has not yet been validated in term burst-suppression EEG.

The mean values of IBIs during the whole recording period of studies I and IV were 8.7 and 7.4 seconds, respectively, in infants with normal follow up. The obtained IBIs are generally comparable with the 6-18 seconds mean IBIs previously reported in healthy preterms of the same GA interval [42, 53, 55, 58]. However, as already pointed out, detailed comparisons may be difficult since studies using manual measurements of IBIs have applied different amplitude and/or duration criteria regarding burst and interburst intervals. This ‘thresholding’ is likely to impact burst/interburst durations similar to the differences between raters in the thesis study II.

In contrast to results from studies using quantitative surrogate measures of continuity (i.e. derived from EEG amplitudes) [85-86], we used EEG measures directly related to preterm developmental physiology and neurobiology, i.e. detection of bursts and IBI (or ‘SATs’ and ‘interSAT’), as suggested by some investigators [47, 86]. It is reasonable to hypothesize that the developmental burst/SATs events and/or IBIs are suitable markers of the brain’s well-being in preterm infants. The duration of averaged IBIs is consequently associated with outcome, as shown in the present studies I and IV. However, the most accurate quantitative EEG measure for prediction in study IV was interburst%, which is the percentage of the recording that is detected as IBI, and thus also reflecting the duration of burst activity. In study II we showed that burst% (complementary of interburst%) is less sensitive to detection thresholds and reflect discontinuity more consistently than the number of, or duration of, bursts and IBI.

The upper and lower borders of aEEG amplitudes in discontinuous traces reflect the amplitudes of bursts and IBI, respectively, but the amplitude of the lower border also reflects the length of IBIs [39]. Many studies in preterm infants have applied manual quantification of aEEG amplitudes, and in study I we found that suppressed aEEG amplitudes were associated with brain damage and handicap. However, the amplitude values obtained by exporting 1-second epochs of aEEG in the NicoletOne software for further analysis are due to the short epoch lengths not comparable to visual analysis.
since these amplitude measures actually represent an unselected mixture of amplitudes during both bursts and interburst. Niemarkt et al used the same software and exported longer epochs (10-15 s) to obtain amplitudes that were more comparable to the visual on-screen appearance of the aEEG trend [96].

**Early aEEG/EEG and brain damage**

Healthy preterm infants seem to have increasing electrocortical activity during the first days after birth, and our results are in line with a few previous reports although none has evaluated this phenomenon specifically [58, 85, 106]. It is difficult to assess whether these early changes represent maturational changes, or a recovery from the birth process. Interestingly, the early improvement of EEG background was present only in infants with good outcome. Hence, absence of background improvement may be indicative of brain dysfunction with long term importance.

A majority of IVHs develop during the first three postnatal days [153], and our findings that IVHs are accompanied by transient aEEG/EEG background depression is also in accordance with previous reports [61, 82-85, 102, 154]. Some investigators have noted that aEEG or EEG background suppression sometimes is observed prior to the time point when brain injury is demonstrated by cUS [68, 82, 85, 103]. If such early markers in the aEEG/EEG could be identified with high accuracy, this would open up for neuroprotective strategies and consequently the time course of injury and the sensitivity/specificity of the above findings have to be further addressed.

The usefulness of EEG for prognostic purposes in newborn infants has been suggested since the 1960’s [155], and both IBIs and other measures of increased discontinuity have been related to adverse outcome in very preterm infants. In 1988 Connell et al reported on the long-term prognostic value of continuous two-channel EEG during the first 72 hours in infants of 34 weeks or less GA [68]. Prolonged IBI was associated with severe IVH and the prognostic values of EEG and repeated cUS were very similar. However, an additional prognostic value of the EEG was noted in infants with IVH grades II-III, since outcome was diverse in these infants and not predicted by cUS alone. In study IV, only presence of IVH grades III-IV predicted outcome, and the aEEG/EEG measures were overall better than cUS in the context of outcome prediction. Visually assessed aEEG continuity during the first three days was likewise associated with severity of IVH and two-year outcome in a cohort of preterm infants with mean GA 25 weeks [61]. In another cohort of very preterm infants with large IVHs, the maximum number of bursts/hour, manually counted in the aEEG trend during the first 24-48 hours
of from birth was predictive of long-term outcome [84]. More recently, both Bowen et al [85] and West et al [86] studied quantitative measures derived from the amplitude of two-channel EEG during the first 48 hours among infants born at less than 29 weeks GA. Bowen et al analysed three 2-h EEG epochs and found that EEG activity <80% at the 10 µV-level was a marker of poor short-term outcome, with PPV 73% and NPV 86% for death or any IVH. West et al assessed 1-hour of aEEG and quantitative measures (activity at 25 µV-level) of two-channel EEG recording in 76 infants and reported PPV 41% and NPV 84% for prediction of poor outcome until a median age of 15 months.

Burst-suppression is associated with poor outcome in term infants. We showed in study IV that presence of BS is associated with poor outcome also in extremely preterm infants. A closer assessment of this predictor shows that the sensitivity for prediction of poor outcome was relatively high (89%), but that specificity was lower (64%), with PPV 68% and NPV 88%. This is in line with overall findings on outcome prediction based on aEEG/EEG in preterm infants where NPVs for prediction of adverse outcome are generally higher than PPVs, i.e. a ‘normal’ aEEG/EEG pattern strongly suggests a good outcome. [66, 85-86, 156].

One reason behind the modest specificity of BS as predictor of poor outcome is that transient BS may develop after administration of medications such as morphine or other opioids, also in preterm infants with good outcome [129]. It was also recently shown that BS patterns in aEEG are common in preterm infants developing sepsis but does not seem to have any influence on long-term aEEG maturation or short term outcome [157]. In the aEEG, a tracé discontinu (TD) pattern shows some variability of the lower border amplitude, while a BS is usually very flat without variation. However, the important distinction between the normal TD [43] in preterm infants and BS may be difficult, especially in the aEEG trend [158]. This is a main reason to repeatingly confirm aEEG classification by inspection of the single-channel EEG.

Seizures and brief rhythmic discharges are markers of abnormal brain function [159]. In previous studies, suspected seizures in aEEG were detected in 60-75% of preterm infants with IVH. [61, 82]. However, presence of suspected seizures was not predictive of outcome in infants with large IVHs [84]. Later aEEG/EEG studies (including study IV) have confirmed the association between brain injury, mainly presence of IVH, and seizures. Shah et al recently presented data on seizures, diagnosed in aEEG tracings with secondary confirmation by simultaneous two-channel EEG, in 51 preterm infants (median recording duration 74 h) [160]. Seizures were identified in 11 (22%) infants and associated with adverse neonatal outcome (death or
abnormal MRI) [160]. However, three out of 11 infants (27%) with seizures had no IVH. In a study by West et al [86], five out of 76 preterm infants had seizures in a 1-hour recording of two-channel EEG, and all five infants had a poor outcome.

A majority of preterm seizures are very brief; mean durations of less than one minute [161] and two minutes [162], respectively, have previously been reported and were also confirmed by the findings in study IV showing a median seizure duration of 60 seconds. The incidence of seizures among infants in study IV, despite the single-channel mode of recording, was 43% which is considerably higher than previously reported [160]. However, no previous study has assessed more than 2600 h of single-channel EEG during the first postnatal days in very preterm infants (median 56 h per infant). Although brief seizure events were common in our population, their presence did not correlate with long-term outcome. About half of the brief seizures (49%) were possible to detect in the aEEG indicating, in line with previous studies [163-165], that the aEEG trend severely underestimates the amount of seizures.

Presence of cyclicity in extremely preterm infants during the first postnatal days is associated with good outcome, as shown both in study IV and in a few other studies [61, 63, 85]. Crude sleep-wake cycling has previously been identified in aEEG and EEG from 25 gestational weeks, although more evident after 27-30 weeks [57, 62-63]. Our findings show that cyclic variations in cortical activity may be visible very early in preterm life, although there is currently no data available demonstrating that this is an immature equivalent of sleep-wake cyclicity. The presence of early preterm cycling activity indicates the importance of considering activity states in the interpretation of even extremely preterm aEEG/EEG.

In summary, studies I and IV were, to our knowledge, the first to demonstrate that quantitative measures of EEG background, continuously monitored over several days, are associated with brain damage and predictive of two-year outcome in VPT and EPT infants. However, the predictive accuracy of the very early aEEG/EEG is moderate and much lower than in full term asphyxiated infants [81]. A probable reason for this is that intercurrent illnesses, e.g. late onset sepsis, and other complications of prematurity may affect long-term outcomes in the preterm infants. Term infants, however, who have recovered from initial illness more often have an uncomplicated and stable course.
Early aEEG/EEG and perinatal inflammation

TNF-α is considered a key cytokine in injury to neurons and oligodendrocyte precursors [30-31]. Data supporting a causal link between inflammatory response and cortical activity is scarce, but experimental studies show that administration of TNF-α to isolated newborn rat myenteric neurons leads to cell membrane hyperpolarization [135]. If similar mechanisms are present within the immature brain this could explain the association between increased levels of TNF-α and the EEG depression observed in our preterm infants in study I. A limitation of the study is the small number of infants. It should be regarded as a pilot study.

Results from studies using isolated hippocampal slices also indicate that glial TNF-α directly modifies synaptic activity through AMPA and GABA circuits [166-168], but the relevance for this action in relation to EEG and preterm brains is not known.

Carbon dioxide effects on EEG background

The results in study III confirm findings of two earlier studies in preterm infants, also showing that increasing blood levels of carbon dioxide are associated with EEG depression [109, 111]. Victor et al only studied infants without large IVHs. We also included data from infants with severe ultrasound abnormalities, but the association between carbon dioxide and electrocortical activity was only present in infants who survived the first week without major brain damage. These findings imply that the EEG response to carbon dioxide, in parallel with cerebral blood flow (CBF) autoregulation, may be disrupted in infants with brain injury [25, 113].

*In vitro* studies verify decreased neuronal excitability at hypercapnia, where decreased field excitatory postsynaptic potentials were found dependent on changes in intra- or extracellular pH, respectively [169-170]. Recently, spontaneous network events in neonatal rat neurons were effectively suppressed by increased carbon dioxide levels. The effect was proportional to, and it was concluded that it was also mediated by, intracellular neuronal acidosis [171]. Even small acid load affected the spontaneous networks events, indicating that neonatal neurons are very sensitive to changes in intracellular pH [171].

It is unlikely that the suppression of cortical activity in study III was mediated by changes in cerebral blood flow. The CO₂-CBF-reactivity will increase CBF with increasing CO₂ [25] while decreased neuronal excitability occurs also in vitro where the CBF variable has been eliminated [172].
Murdoch Eaton et al reported that both respiratory and metabolic acidosis was associated with EEG changes [109]. In contrast, Victor et al found no relation between blood pH and EEG. Experimental data show that hydrogen ions not readily diffuse over the blood brain barrier [173] and carbon dioxide may thus have larger effect on cerebral pH and neuronal excitability. We found in study III that pH was to 80% determined by PaCO$_2$. We had very few samples with metabolic acidosis, and since base deficit is a calculated value, rather than directly measured, we abstained from analysing effects from metabolic acidosis on EEG. However, if the EEG suppression is mediated by cerebral pH it would be highly relevant to further study the duration of EEG depression at hypercarbia, since neuronal intracellular pH is likely to be rapidly corrected [169] and changes in excitability may therefore depend on both the extent of carbon dioxide fluctuation and the rate of CO$_2$ change.

Permissive hypercapnia is commonly used as a lung protection strategy during mechanical ventilation of preterm infants. By acceptance of mild to moderate hypercapnia (6-7 kPa) high tidal volumes and pulmonary over distension can to some extent be avoided. A certain degree of hypercarbia may also reduce the risk of unintended hypocarbia which is associated with risk of WMD [174]. Several RCTs and retrospective studies have investigated the safety of permissive hypercapnia and found no increased risk of IVH or adverse neurodevelopmental outcome related to hypercarbia [175-177] although one study indicated that large fluctuations of PaCO$_2$ were predictive of lower BSID results [178]. The increased discontinuity at high PaCO$_2$ (and high plasma glucose) levels should be interpreted as a sign of functional neuronal impairment rather than established neuronal damage, at least initially but any neurodevelopmental consequences of the association between hypercapnia and EEG suppression is currently not known. These findings emphasize the monitoring properties of the single-channel aEEG/EEG as compared to its use for diagnostic or prognostic purposes.

Glucose effects on EEG background

The depletion of substrate needed to maintain neuronal function may well explain any EEG depression during hypoglycaemia. In rodent hippocampal neurons, glucose depletion effectively blocked spontaneous network events [171]. However, neurophysiological evidence demonstrating that hypoglycaemia adversely affects brain function or EEG in newborn infants is, as already discussed, very limited [123-124]. Stenninger et al evaluated effects of hypoglycaemia on the aEEG in 12 term infants of diabetic mothers. Hypoglycaemia (mean blood glucose 1.5 mmol/L) was associated with a subtle, but statistically significant reduction in maximum aEEG amplitude [124].
Furthermore, a few case reports have shown transient flattening of the aEEG during severe hypoglycaemia [39]. In contrast, hypoglycaemia was not associated with measurable changes in various EEG parameters (aEEG minimum amplitude and continuity, and spectral edge frequency) in newborn lambs and in preliminary data from newborn infants above 32 gestational weeks [125, 179]. Moderately preterm and term infants, as well as newborn lambs, have more continuous EEG than the extremely preterm infants in the present study. Consequently, measures of EEG continuity (such as IBI) may be more sensitive for evaluating preterm infants than term infants. Furthermore, given the normal EEG discontinuity in preterm infants, measures of continuity may be more directly correlated to brain function than power measures. Comparable to the studies by Harris et al, we did not find any changes in relative band power related to glucose levels, indicating that we would also not have been able to demonstrate major changes in spectral edge frequency.

Hyperglycaemia is common in preterm infants where immature physiology and need for parenteral nutrition predispose for high plasma glucose concentrations. No previous studies have, to our knowledge, investigated possible EEG changes during neonatal hyperglycaemia. Hyperglycaemia might act as an oxidative factor with negative effects on brain cells, especially oligodendrocytes, which may be an important explanation of the long term consequences of hyperglycaemia [120], but unlikely of any importance as concerns rapid fluctuations in cortical activity along with changes in plasma glucose.

Skov and Pryds measured cerebral blood flow (CBF) in preterm infants with low blood glucose and found a rapid decrease in CBF after i.v. infusion of glucose [180]. They suggested the presence of a central glucose sensor which increases CBF at low glucose levels in order to provide sufficient amounts of substrate for brain function. Such mechanisms may explain the relation between moderate hyperglycaemia and EEG continuity in study III. The present findings are also supported by preliminary data in a group of 24 infants with GA <28 weeks where higher blood glucose concentrations during the first three days were associated with suppressed aEEG background [181].

A limitation of study III is that data consist of time series, but not with a natural course. Carbon dioxide and plasma glucose were measured on clinical indications and actions were taken to correct values towards the intended ranges. This compresses the range of observations around values that have been regarded as ‘optimal’. Samples therefore include relatively few very low or high values of both carbon dioxide and plasma glucose, and consequently the results should be interpreted with caution outside the clinically intended ranges. Any interaction between the effects of carbon dioxide, pH
and plasma glucose on the immature brain may be further investigated. Concerning plasma glucose it could be speculated that very high glucose concentrations may indicate a more severe general illness, which in turn may conceal physiological associations between glucose and electrocortical activity.

Improving long term monitoring of EEG in preterm infants

Artefacts are common in neonatal aEEG/EEG monitoring, and automated EEG analysis may be sensitive to such artefacts, e.g. movements or care procedures [79-80]. Some artefacts may be compensated for in automated algorithms, for example by baseline subtraction [182]. Seizure activity might also interfere with automated analysis such as burst detection, because both seizures and normal bursts have multifrequency, complex waveform patterns. In the present studies II, III and IV, epochs containing seizure activity were excluded from analysis. In future neonatal brain monitors, coincident and independent detection of seizures should be included in addition to background measures.

There are currently several lines of ongoing research and development of tools for automated background continuity analysis [100, 182-183]. The optimal strategies (for today’s technical solutions) may vary depending on aim: identifying BS or DC patterns, in contrast to continuous activity [184], segmentation of BS patterns in asphyxiated term infants [100], or quantification of discontinuous patterns in preterm infants [182]. For clinical purposes the ideal automated segmentation algorithm detector should have both diagnostic (regarding acute brain damage) and prognostic value. Current literature, including the first and last study of this thesis, suggests that quantification of bursts or IBIs in the youngest preterm population can provide valuable information about both diagnosis and prognosis [68, 84]. For the future, improvements in burst/SAT detection may be achieved by development of algorithms independent of absolute EEG amplitude thresholds [182]. For example this could be done by employing supervised machine learning techniques where patterns are ‘learned’ through extraction of suitable classification features from samples of manually classified training data [100]. Ideally, the detector would be capable of distinguishing bursts/SATs from the ongoing oscillations that increase with GA in the preterm EEG.

A further question, partly addressed in study IV, is which indices derived from burst detection that gives the most valuable clinical information over longer periods of time (e.g. number of bursts, burst durations, IBIs, burst%, interburst%). Also, the statistical measures of burst occurrence (e.g. mean,
median, percentiles or variability measures), with the highest discriminatory properties for identification of abnormal brain activity have to be evaluated. In clinical use, the different combinations of predictive values, sensitivity and specificity of different methods must be balanced against the intended aims: High sensitivity measures will be needed for identification of infants at high risk of brain damage, where more specific investigation and closer surveillance may then be initiated. Further, high NPV of EEG measures may put more confidence behind a reassuring message in the situation of parent’s counselling when EEG indicates no abnormality. Additionally, any active intervention with potentially serious adverse effects will rather demand a high PPV and high specificity when unfavourable EEG measures are identified. Also when non-survivors were excluded from analysis of study IV, the early aEEG/EEG (but not CRIB-II score or cUS findings) differed between infants who developed NDI and infants who had a normal outcome at two years. Beside this important indication on long term importance in comparison with standard clinical data, the overall gain in predictive values from single-channel EEG compared to a combination of clinical data in the CRIB-II was small. In clinical use, the importance of improved prognostication must be balanced against any potential harm from the introduction of additional procedures in the care of extremely preterm infants.

During early brain development, bursts/SATs are first focal events [47]. Hence, single-channel recordings may have low sensitivity to detect early SATs, and cannot assess the extent to which cerebral cortex is engaged by a single event. Also, burst waveforms in single-channel recordings depend on the locations of cortical events in relation to the electrode position. The amplitude of some bursts is, for example, much higher than others just because of the distance to the recording electrode. This will together affect both visual and automated burst detection. Therefore multi-channel EEG may be the choice of basic research on burst/SAT occurrence in relation to brain development. However, for clinical purposes it is reasonable to expect an acceptable trade-off with single-channel EEG in extremely preterm and extremely vulnerable infants.

The optimal number of electrodes for seizure identification in preterm infants is also unknown. A single- or limited-channel montage will not be able to detect all seizure activity [41, 158, 185]. Several authors have shown that about 80% of all seizures may be identified in aEEG with guidance of single/two-channel original EEG [41, 158, 186]. The aEEG trend in itself (without guidance of original EEG) on the other hand, seems to severely underestimate seizure occurrence [163-165], which is also very obvious from the present study IV.
Study IV addressed the clinically important questions of how (intermittently or continuous) and when (how early) to record single channel EEG/aEEG in extremely preterm infants. The time restricted (1-hour) recordings without any knowledge of the infant’s status, had low artefact burden as compared to the bulk of long term recordings and were chosen so that no morphine had been administered during the last three hours. The equal predictive value of short but low artefact recordings and data obtained by long term monitoring possibly suggests that detailed analyses of repetitive and attended conventional EEG should be performed for the purpose of optimal prediction. The feasibility of full-scale conventional EEG in early neonatal care can however be seriously questioned and detailed analysis of extremely preterm conventional EEG is certainly an expert issue.

Also, the value of very early (within hours from birth) aEEG/EEG is important to assess in high-risk very preterm infants. In study IV significant differences in background activity between infants with good and poor outcome were at first identified very closely before 24 hours age, although some studies report differences between infants with normal and adverse outcome also during the first postnatal day [71, 85]. Accordingly, it is important to further investigate the value of very early recordings as our results do not support the use of aEEG/EEG for predictive purposes on the first postnatal day in vulnerable extremely preterm infants.

Finally, I want to call attention to the separate purposes of EEG/aEEG recording: for prognostic use time-limited recordings of high quality may work well, but as indicated by study IV, still be insufficient for detection of seizure activity. Detection of developing background abnormalities in real-time, related to unexpected events also demands long-term monitoring.
Conclusions

The presented studies demonstrate that:

Automated NLEO-based detection of bursts or IBI has acceptable discriminatory properties and clinical relevance making it a useful scientific tool and a promising technique for long term monitoring of discontinuous EEG background.

The aEEG trend and quantification of IBI in single-channel EEG provide moderate prediction of outcome in extremely preterm infants already after 24 postnatal hours, and also in the absence of IVH.

Awareness of recording quality is very important in single-channel aEEG/EEG analysis.

Background suppression according to single-channel aEEG/EEG monitoring is an early marker of brain damage.

Fetal inflammatory response, as signalled by increased level of umbilical cord TNF-α, may be associated with acute depression of neural function detectable in early EEG background.

Increasing EEG discontinuity occurs at carbon dioxide levels often accepted as part of lung protection strategies.

Plasma glucose concentrations are also associated with electrocortical activity in extremely preterm infants.

The results may, in the future be used for early identification of infants at high risk of brain damage and adverse outcome and could also have implications for early intervention.
Future perspectives

Monitoring brain function by aEEG/EEG may, already today, aid immediate detection of altered cerebral function secondary to other physiological disturbances. In this way monitoring may guide the care and indicate need for closer surveillance or specific investigations. To extend this possibility in preterms, more knowledge of specific pathophysiological and pharmacological effects on EEG background is needed. Furthermore, automated seizure detection may also be valuable tools for early detection of brain dysfunction.

The possibilities of direct action targeting the harmful processes behind preterm brain damage and neuro-developmental impairment are small today. Early intervention programs for preterms seek to take advantage of the plasticity of the immature brain, in order to compensate for established damage [187]. In the future however, specific neuroprotective interventions may be developed and hence, also the possibilities to act when an unfavourable condition is recognized. In this perspective, further development of brain monitoring and better methods for prediction of neurodevelopmental outcome in preterm infants will be vital for clinical action. Specifically, detection of neural dysfunction reflecting yet not established brain damage is requested. The neurophysiological time course of brain lesions should consequently be further addressed in this population, with focus on the early time-limit of prediction - when there still is time to act. Prognostic applications of early preterm aEEG/EEG are however, less probable to have implications on end-of-life decisions (withdrawal of care) in preterms as compared to asphyxiated term infants where the prognostic value is considerably higher.

From a future psychiatrist’s point of view, enhancement of early infant-parental attachment is interesting. In situations where parents trust and hope for the future of their child (and in worst case also their early psychological attachment) is failing, a reassuring counselling may be confidently based on prognostic favourable EEG findings when available.
Avhandlingsarbetets övergripande syfte var att karaktärisera en-kanaligt EEG och amplitudintegrerat EEG (aEEG) hos mycket för tidigt födda barn i relation till hjärnskador, utveckling och fysiologi. I fokus var kvantitativa mått i långtidsövervakning av det omogna barnets diskontinuerliga elektrokortikala bakgrundsaktivitet.

I arbete I undersöktes om kvantitativa mått i tidigt aEEG/EEG hade samband med strukturella hjärnskador synliga på ultraljud, utveckling vid två års ålder eller perinatal inflammation. Sexton barn med genomsnittlig gestationsälder på 25 veckor ingick i studien. EEG’ts interburstintervall (IBI) var längre hos barn med neonatala hjärnskador och hos barn som utvecklade handikapp än hos friska. TNF-α i navelsträngsblod korrelerade med IBI.

I arbete II undersöktes överensstämmelse mellan bedömare vid visuell detektion av “burstar” i EEG. Detta jämfördes sedan med en algoritm för automatiserad analys av IBI. Datamaterialet bestod av 10-minutersavsnitt av en-kanaligt EEG från tolv extremt prematura och sex mycket prematura barn. Tre oberoende bedömare markerade “burstar” i EEG’t med en “overall-agreement” punkt-för-punkt på 86% hos de extremt prematura och 81% hos de mycket prematura barnen. Sensitiviteten hos detektionsalgoritmen var 64% för extremt prematura respektive 69% för mycket prematura barn. Specificiteten var 96% respektive 88%. Algoritmen modifierades i nästa steg med syfte att förbättra precisionen.

I arbete III undersöktes om arteriella koldioxidnivåer (PaCO₂) respektive plasmaglukos korrelerade med kortikal bakgrundsaktivitet 32 extremt prematurfödda barn. Totalt analyserades 247 blodprover med avseende på koldioxidnivå och glukoshalt, och simultant med detta kvantifierades IBI i enkanaligt EEG. Vi fann att längden på IBI ökade med stigande PaCO₂. Detta samband förelåg dock endast hos barn som överlevde den första veckan utan hjärnskada. Vid ett dygnstående ålder innebar 1 kPa ökning i PaCO₂ en förlängning av IBI med 16%. Korrigerat för koldioxidnivåer uppvisade plasmaglukos ett U-format samband med IBI som var kortast vid plasmaglukos på 4 mmol/l.

I arbete IV undersöktes det prediktiva värdet av tidigt en-kanaligt aEEG/EEG. Både visuell skattning och kvantitativ analys av EEG utfördes. I
studien inkluderades 41 barn med gestationsålder 22-27 veckor (extremt prematurfödda) och 8 barn med gestationsålder 28-30 veckor (mycket prematurfödda), och dessa följdes upp med utvecklingsbedömning hos psykolog och läkare vid två års korrigerad ålder. Förekomst av burst-suppressionmönster, förekomst av kontinuerlig bakgrundsaktivitet och förekomst av sömn-vakenhetscykler identifierades som prognostiskt betydelsefulla i visuell bedömning av aEEG-trenden. Kvantifierade interburstintervall och interburst-% (den andel av registreringen som utgörs av IBI) var även de prediktiva för två-årsutfall och interburst% hade studiens högsta kombinerade sensitivitet och specificitet. Epileptiform aktivitet sågs hos 21 barn (43%) och var vanligare hos barn med påvisbar hjärnskada. Sådan anfallsaktivitet hade dock ingen koppling till handikapp vid två års ålder.

Sammanfattningsvis visades att bakgrundsaktiviteten i enkanaligt aEEG/EEG är en tidig markör för hjärnskada och prediktiv för utveckling till två års ålder hos extremt för tidigt födda barn. Den elektrokortikala bakgrundaktiviteten uppvisar även samband med perinatal inflammation samt blodets koldioxidnivåer och glukoshalt. Den testade detektionsalgoritmen för automatiserad mätning av interburstintervall har prestanda som gör den användbar i klinisk forskning. De aktuella resultaten gör det även troligt att kvantitativ analys av det prematura EEG’ts bakgrundsaktivitet (så som bestämning av IBI) kan bli kliniskt användbara.

En utvecklad övervakning av det prematurfödda barnets hjärnaktivitet kan i framtiden bli användbar i syfte att tidigt identifiera barn med hög risk för hjärnskada och utveckling av handikapp. Att identifiera dessa barn kan idag ha betydelse för tidig riktad utredning men framgent kan detta även få betydelse för aktiv intervention, om möjligheterna till specifik neuroprotektion utvecklas.
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