EEG and spectral edge frequency: analysis in posthypoxic newborn piglets

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Abstract

OBJECTIVE: To evaluate the frequency content of the electroencephalogram (EEG) during recovery after a severe hypoxic insult in newborn piglets.
METHODS: EEG was continuously monitored in nine newborn piglets exposed to a severe hypoxic period. Power spectra in five frequency bands were calculated using Fourier transformation. Spectral edge frequency 90 (SEF90) was defined as the frequency below which 90% of the power in the EEG was located. The piglets were divided into two groups; Group 1 represented piglets with some EEG recovery and Group 2 represented piglets without any EEG recovery.
RESULTS: The recovery of the EEG in Group 1 had the same time course in all frequency bands. SEF90 indicates recovery earlier than the value of total power. But SEF90 also signals activity in the EEGs that were almost completely suppressed. When SEF90 was calculated during periods of periodic EEG activity during the very early phase of recovery, the values fell within the same range as during the control period.
CONCLUSION: Spectral analysis of continuous EEG in newborn piglets exposed to very severe hypoxia showed that no specific frequency band of the EEG preceded the other ones during recovery. The results of the SEF90 measure, demonstrates the need for critical analysis of the raw EEG before any reliable estimation of cerebral function can be made.

Abbreviations :

SEF90	- Spectral edge frequency 90
EEG	- Electroencephalogram
MABP	- Mean arterial blood pressure
ECG	- Electrocardiogram
FIR	- Finite impulse response
SACS	- Signal Archiving and Communication System

ORIGINAL ARTICLE

INTRODUCTION

Perinatal hypoxic-ischemic events remain a major cause of permanent brain injury in the full term newborn infant. The recent development of therapeutic possibilities such as hypothermia (Perlman 2006; Thoresen & Whitelaw 2005) and pharmacological therapy (Peeters & van Bel 2001; Zhu *et al.* 2009) has underlined the need to recognize patients at a very early stage who would benefit from interventions and also to detect those who should not be exposed to this kind of therapy. Several studies have reported that EEG can be used as a predictor of long term neurodevelopmental outcome after such an event (Biagioni *et al.* 1996; Holmes, Lombroso 1993; Pressler *et al.* 2001; Selton, Andre 1997; Wertheim *et al.* 1994). In the neonatal intensive care unit EEG is rarely used in an emergency situation, both because of difficulties in online interpretation, and because of practical problems in the bedside situation. This has led to the development of EEG derivatives that can be interpreted by clinicians bed side. One such measure is spectral edge frequency (SEF) where a frequency level is chosen below which a certain percentage of the EEG energy is situated (Inder *et al.* 2003). SEF90 has been advocated as a monitoring facility for brain function in premature human neonates (Inder *et al.* 2003). However, previous studies have indicated that various frequency bands of the EEG react differently during oxygen lack (Goel *et al.* 1996; Guthrie *et al.* 1982; Symmes *et al.* 1970).This would affect the SEF in a systematic way. The primary aim of this study was to characterize the reappearance of the complex EEG signal during recovery from severe hypoxia.

We used the original EEG signal to evaluate the frequency content of the signal during the recovery phase after a standardized hypoxic insult, severe enough to cause isoelectric EEG and high risk of permanent brain injury (Thoresen *et al.* 1996). In order to study an experimental animal where the maturation of the brain is similar to term human neonates, newborn piglets were used (Mattson *et al.* 1978; Sävman *et al.* 2005; Thoresen *et al.* 1996).

METHODS

Animal preparation

Nine newborn piglets were used in the experiment (median weight 1.5 kg, range 1.0–2.3 kg; median age 2 days, range 12 h–4 days). Anesthesia was induced with ketamin 30 mg/kg i.m. and azaperone 4 mg i.m. The anesthesia was maintained with N_2O in the inspired gas and chloralose 25 mg/kg i.v. followed by intermittent bolus doses of 15 mg/kg i.v. to keep the piglet sufficiently sedated. The piglets underwent tracheostomy and central arterial and venous lines were placed. They were mechanically ventilated (Baby log 8000, Dräger) using a mixture of 30–35% oxygen, 30% N_2O and nitrogen gas. During baseline and recovery periods the ventilator setting was adjusted to target a transcutaneous arterial oxygen saturation (S_aO_2) >96% on a pulsoxymeter probe placed on the hindleg. Arterial CO_2 tension was kept at 4.0–5.5 kPa at all times. Three lead electrocardiogram (ECG) and intra arterial blood pressure was continuously measured, using a Grass Polygraph, model 7B. Mean arterial blood pressure (MABP) was targeted to be above 30 mm Hg . To achieve this, one piglet needed a short course of dopamine; dose 2.5–17 µg/kg/min. Deep rectal temperature was continuously measured and kept at 38.5–39.5 °C by means of a heating pad and a heating lamp. Arterial blood gases, glucose, electrolytes and lactate levels were measured intermittently using a Radiometer ABL 725. Infusions of glucose solutions, 100 mg/ml, were given in varying amounts to keep the blood glucose level between 4–10 mmol/l. At the end of the experiment, the animals were put to death with a lethal dose of an anesthetic agent (pentobarbital).

EEG recording and preprocessing EEG was recorded from two bipolar silver rod electrodes, 3 mm in diameter, snugly fitted through burr holes in the bone and placed on the dura. The electrodes were symmetrically placed, 15 mm lateral to the sagittal suture and 10 mm dorsal to bregma. The system used for recording, SACS (Signal Archiving and Communication System), is a software system which runs on a standard PC under Windows operating system (Lindekrantz *et al.* 1999). The signals were band pass filtered (1.5–44 Hz), amplified by an analogue amplifier and stored digitally using a sample frequency of 100 Hz. Most recordings were found to be contaminated with electrocardiogram (ECG), power-line interference and breathing interference, which was problematic, especially when EEG amplitudes were low during hypoxia and the recovery period. The ECG interference was reduced by using a finite impulse response (FIR) adaptive filter (filter length Fs/2, step size 2/Fs) (Hayes 1996). This filter subtracts a filtered ECG from the EEG, where the filter parameters are adapted to minimize the correlation between the ECG and the EEG. Therefore, this filter has little effect on the true underlying EEG spectrum. The breathing interference was narrow-banded and stable because a ventilator had been used. Due to these circumstances, the breathing artefacts could efficiently be reduced using a notch filter at the breathing frequency. Since the filter was designed to be very narrow banded, it did not appreciably affect the EEG spectrum. (The frequency bands used in the frequency analysis described below are much wider than the narrow band filtered out). The power line interference was handled in the same manner and the effect from the filter used for this is also negligible.

All filtering affects the signal, and has to be done with care. The filters we have used have as little impact on the true EEG as possible and signals have been analysed in both frequency and time domain after filtering to ensure that the filters did not cause any unwanted effects.

Protocol

The experiment largely followed the protocol described by (Thoresen *et al.* 1996) with modifications mainly of the anesthesia and ventilatory strategy, allowing adjustments to keep $PaCO_2$ stable throughout the experiment. The piglet was placed in prone position. After stabilisation, a 60 minutes baseline registration was performed. Hypoxia was then induced by reducing F_iO_2 to 6% for 45 minutes (60 minutes for two piglets). After a hypoxic period of 60 minutes two of the piglets had no EEG recovery. We therefore reduced the hypoxic period for the remaining piglets to 45 minutes. When the amplitude of the EEG decreased below 20 μV, oxygen concentration in the inspired gas mixture was gradually increased up to at most 8% to maintain a barely visible EEG activity during the period of hypoxia. The ability of the cardiovascular system to tolerate the reduced F_iO_2 varied: When severe bradycardia (defined as heart rate < 100/min) and/or severe hypotension (defined as MABP < 25 mm Hg) occurred, the F_iO_2 was transiently increased until the heart rate and blood pressure recovered, whereupon oxygen was again reduced according to the tolerance of the animal. The insult was terminated followed by reoxygenation with 50% oxygen for 30 min. Thereafter F_iO_2 was reduced to 30% or to the minimum level producing $S_aO_2 > 96\%$. The piglets were monitored up to 18 hours after the insult. The protocol was approved by the ethics committee of the University of Göteborg.

Analysis

All EEGs were visually analyzed by a neurophysiologist (MT), specialized in newborn EEG interpretation to exclude periods of recording with artefacts. Power spectra were estimated using the standard mathematical software Matlab by applying the Fourier transform on detrended 2 s segments using a Hann window. The recovery period after the hypoxia was analyzed regarding the power within five frequency bands; < 2 Hz, 2–4 Hz, 4–8 Hz, 8–13 Hz, 13-30 Hz and also for the total power (< 30 Hz). The spectral power per frequency band was expressed in relation to a reference spectrum, which was calculated for each piglet by averaging over 15 minutes baseline registration obtained immediately before the start of hypoxia. Spectral edge frequency 90 (SEF90) was defined as the frequency below which 90% of the power in the EEG was located. Total power was defined as the spectral energy content below 30 Hz. Serial episodes of 60 seconds were analysed along the recovery period for each piglet; every 2 minutes for the first 2 hours, every 15 minutes for 2 hours, every 30 minutes for 2 hours and thereafter once every hour until the recording was ended. The animals were divided into two groups.

Group 1 comprises four piglets with some EEG recovery (reappearance of continuous EEG) after the insult and Group 2 comprises five piglets without any recovery (EEG was almost completely suppressed and only regained very sporadic abnormal activity during the entire recording period of several hours). The spectral analysis of the piglets in Group 1 was started when the EEG showed a continuous pattern. The time for this differed among the four piglets. One piglet produced such a pattern directly after hypoxia. The other three regained a continuous pattern from 20 minutes up to 2.5 hours into the recovery period. In Group 2 the spectral analysis started directly after the end of the hypoxic episode to study the effects of applying this method on a non continuous signal.

RESULTS

The focus of interest in this study is the recovery phase of the EEG after the start of reoxygenation. The results are presented in graphic form as the power within a given frequency band and as SEF90 respectively. Even though continuous recordings were performed up to eighteen hours after the start of hypoxia no major changes occurred later than after six hours. Therefore, the graphs cover the six hours after the respective start of analysis in the two groups (Figures 1 and 2). In Group 1, the change of power of the EEG within all frequency bands had similar time courses: The signal reappeared gradually (Figure 1). The power content of the EEG showed a slow increase during the ensuing hours (with the exception of one piglet, which did not show recovery of the power content the first six hours despite of a continuous signal). The lower frequency bands, (< 2 Hz and 2–4 Hz) where most of the power in the neonatal EEG is located, did not recover faster and appeared to contain less relative power than the higher frequency bands. When the SEF90 is compared to total power a different time course appears (Figure 2). SEF90 appears to signal recovery earlier than the mean total power (Figure 2C). But the SEF90 also signals activity in the five pigs where the EEG continued to be almost completely suppressed during the entire phase of reoxygenation (Figure 2B). Moreover, when SEF90 was calculated from periodic EEG activity during the very early phase of recovery in Group 1, the values fell within the same range as during the control period (Figure 2D).

DISCUSSION

Several previous studies have examined the frequency spectrum of the EEG during experimental hypoxia. Studies, using newborn primates and fetal sheep (Guthrie *et al.* 1982; Symmes *et al.* 1970) demonstrate a general slowing of the EEG during hypoxia with loss of high frequencies as the initial alteration. Another report has modified this description and instead shown a relative increase of power in medium and high frequency components of the EEG during hypoxic stress in one week old piglets (Goel *et al.* 1996). It appears that both the degree of maturation of the brain and the severity of the

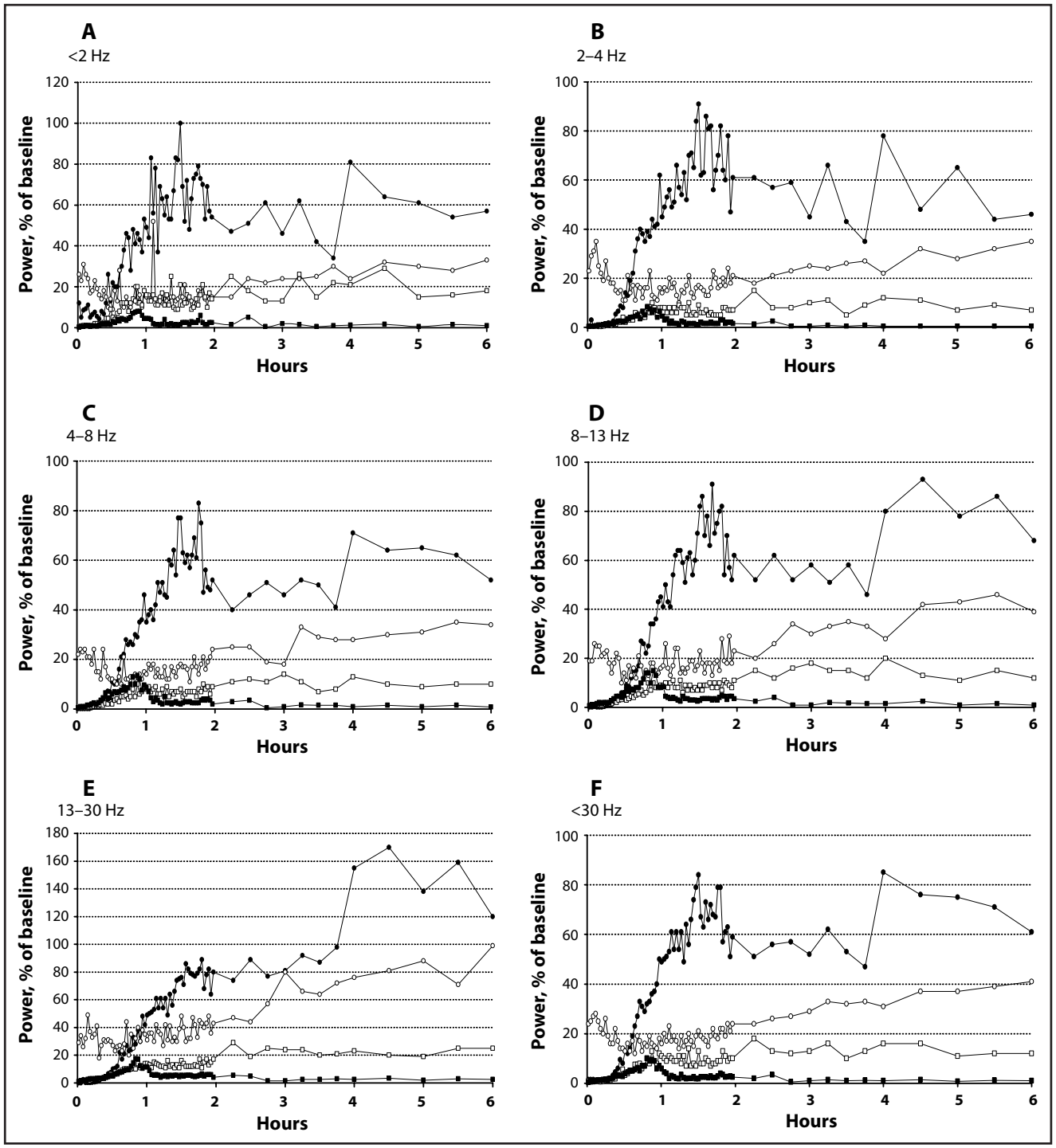

Fig. 1. EEG power in the five frequency bands investigated (A–E) and total power (F) in relation to baseline levels, during recovery in Group 1. Each symbol represents an individual (four piglets).

lack of oxygen will be reflected in the electrophysiological behaviour of the brain during and after the insult. Our experimental model was chosen to correspond to the clinical situation of a term human neonate who has suffered from such severe birth asphyxia that there is a high risk for permanent brain injury in surviving individuals. Therefore, newborn piglets were exposed to periods of hypoxia producing a severely suppressed EEG for 45–60 min. This gives a high probability of severe brain damage (Thoresen *et al.* 1996). The fact that more than half of the animals did not regain any EEG activity during the period of reoxygenation underscores the severity of the hypoxic insult. The recovery of the EEG in those animals that regained EEG activity had the same time course for all frequency bands studied. Thus, when the electric activity returned from

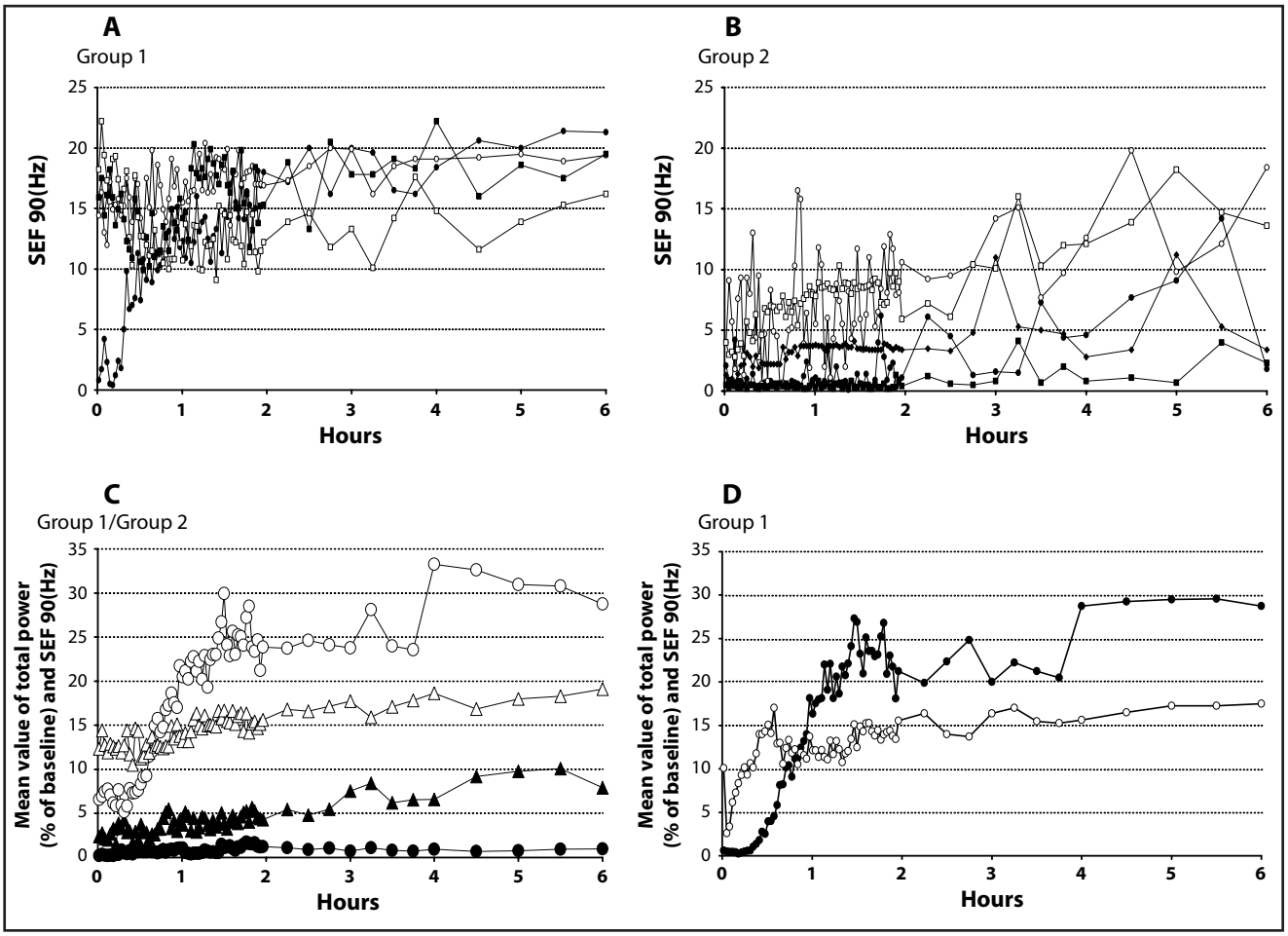

Fig. 2. The development of EEG parameters during recovery. **A, B:** Individual values of SEF90 in the two Groups. **C:** Mean Group values , of the total power (circles) and SEF90 (triangles). Open symbols for Group 1 and filled for Group 2. **D:** Mean total power and SEF90 (filled and unfilled circles respectively) in Group 1, evaluated directly after the end of the hypoxic episode i.e. including the period before the reappearance of continuous EEG . The mean SEF90 during the reference period is also included on the y-axis (time zero).

the severely suppressed state during reoxygenation, no specific segment of the frequency spectrum preceded the others. The SEF90 on the other hand started to climb back to prehypoxic levels already after the first few minutes, also in instances when the EEG had a severely pathologic (mainly suppressed) appearance (Figure 2D). This activity of the SEF90 demonstrates the risk of misleading results when using SEF alone as a measure of the EEG frequency and power distribution (Inder *et al.* 2003). The main reason for this is probably that SEF is a measure of relative distribution of frequencies, regardless of energy content of the EEG. This means that the SEF when applied to *e.g*, a periodic EEG, may produce spurious results. The interpretation of SEF90 values thus depends on the availability and validation of the underlying EEG signal.

In summary: When newborn piglets were exposed to a period of very severe hypoxia, the EEG did not recover in half of the cases while in the other half no specific frequency band of the EEG preceded the other ones during recovery. The SEF90 value did not contribute any extra information in cases with continuous EEG signal while spurious results were obtained in situations with intermittent or very suppressed EEG activity. This underlines the need for critical analysis of the raw EEG before any reliable spectral estimation can be made.

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