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Functional analysis of the neonatal brain

PhD thesis

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2. List of Abbreviations

BAEP- Brainstem Auditory Evoked Potentials

DTI – Diffusion Tensor Imaging

ERP – Event Related Potential

GA-Gestational Age

INC - MRI Compatible Incubator

IVH - Intraventricular Haemorrhage

PHH - Posthaemorrhagic Hydrocephalus

CNS - Central Nervous System

MRI - Magnetic Resonance Imaging

fcMRI – functional Magnetic Resonance Imaging

PVL – Periventricular Leukomalacia

GMFCS- Gross Motor Function Classification System

KABC-II - Kaufmann's Assessment Battery for Children

PDI - psychomotor developmental index

MDI - Mental developmental index

MMN – Miss Match Negativity

CP – Cerebral Palsy

SEP – Somatosensory Evoked Potentials

VEP – Visual Evoked Potential

NICU – Neonatal Intensive Care Unit

ROP – Retinopathy of prematurity

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PDA – Persistent ductus arteriosus

NEC – Necrotic Enterocolitis

CLD – Chronic lung disease

AIS – amniotic infect syndrome

RDS – Respiratory distress syndrome

TOD – Thalamo-occipital distance

AHW – Anterior horn width

BPD – Bronchopulmonary Displasia

PHHN – Persistent Pulmonary Hypertension

HIE – Hypoxic Ischaemic Encephalopathy

NIDCAP -Newborn Individualized Developmental Care and Assessment Program

INC – MRI-compatible Incubator

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3. Introduction

3.1. Challenges of neonatal care

Premature birth presents enormous complexities for all to consider, especially for expectant families. Survival rates of this population has dramatically increased in the last decades, although enthusiasm for this improvement is tempered by the long-term follow-up experience with these children. Despite a significant decline in mortality, neurodevelopmental injury rates remain high and do not seem to be consistently improving. [1, 2]

Follow-up examinations of surviving infants born at less than 27 weeks' of gestation from different institutions and populations demonstrate outcomes that seem to be remarkably consistent. Approximately 25% of infants suffer severe neurologic damage, while 25% has moderate impairment, and 50% are judged to be mildly impaired or normal. [3] These investigations suggest the earlier the gestational age, the higher the disability rate. Furthermore, “normal” surviving infants are at considerable risk for a variety of neurobehavioral, social, and educational deficits that likely reflect altered neurobiology related to premature birth. [4] There is also a growing evidence that late preterm birth also carries a considerable risk for altered neurological outcome. [5]

The challenge of current neonatal care is to improve neurodevelopmental outcome. To achieve this goal neuromonitoring and neuroimaging methods must be regularly used and further improved in order to optimise therapeutic efforts of this fragile population.

3.1.1. Development of perinatal care

In the last decades there is a growing effort that premature birth should take place at centralised level 4 perinatal centers all around the world. Obstetritians and neonatologist have worked out protocolls to protect the fetus and the mother in case of threatening preterm labour. Intrauterine transport, tocolisys with beta-mimetics, magnesium-sulphate or oxytocin-antagonists, cesarian section and antenatal steroids are routine protocolls of level 4 neonatal centers. [6]

In case of extreme premature birth a general agreement is also evident even for week 23 to 25 weeks of gestation: antenatal steroids are recommended, prenatal transport and cesarean section are also indicated to protect the fetus, and resuscitation is offered to all infants without fatal anomalies. In most guidelines for extremely premature birth, the gestational age is considered to be the best estimate of the infant's maturation, and consequently, his or her possibility of survival, although many other fetal/neonatal characteristics could play a role in the prognosis. [7]

A single course of antenatal corticosteroids given 24 hours to 7 days before birth to women in preterm labor at less than 34 weeks' gestation improves lung maturity and reduces neonatal problems, including respiratory distress syndrome, necrotizing enterocolitis, severe intraventricular hemorrhage, and death. [8] Exposure to antenatal corticosteroids was associated with lower mortality or neurodevelopmental impairment at 18 to 22 months in infants born at 23 to 25 weeks’ gestation. However, even though intact survival doubled with the administration of antenatal steroids in the entire cohort, it still remained relatively low (36%).

Regular ultrasound scans especially at 13 and at 20 weeks for genetic anomalies are very important in the management of pregnancies. For further analysis there are fetal MRI sequences that can further specify the fetal developmental problem and provide exquisite data for survival rates and parental counceling. [9]

Other Biomarkers such as Low levels of maternal serum PAPP-A, free β-hCG and increased fetal NT are associated with increased fetal death and genetic anomalies and are part of the routine or obtional screening in different countries. [10]

3.1.2. Development of clinical practice at the neonatal ward

There has been an immense effort to develop new strategies in the clinical care of extreme premature infants. New drugs have been tested such as sildenafil, new methods of ventillation are taking over, such as minimal invasive positive airway pressure and new ways of surfactant administration are introduced. [11] Trials that evaluate neurodevelopmental outcome are providing important data regarding safety and efficacy of NICU treatment strategies. Although hypotension is a risk factor, we do not know what

constitutes hypotension in extremely preterm infants and whether treatment with inotropes or hydrocortisone influences neurodevelopmental outcomes. [12] Different methods of ventilation, positive airway pressure or administration of surfactant via intratracheal tube with or without sedation have been shown to have an effect not only on mortality and morbidity such as the toccurenece of BPD or IVH, but also on long-term neurodevelopmental outcomes as well. [13] The successful usage of nitric oxide in respiratory failure and pulmonar hypertension is widely accepted in everyday clinical practice, but long term prospective randomised data suggest that it should be used only in specificly selected populations. [14] Becuase of some adverse effects with inhaled nitric oxide, prospective studies are needed to judge its effect, especially in combination with sildenafil (a phosphodiesterase (PDE) inhibitor with their potent vasodilator properties) as they might affect the diffusion and effectiveness of NO in persistent pulmonary hypertension (PPHN) [15]

Other nursing strategies are aviable, such as kangoroo mother care that proved to be safe and effective in improving weight gain, mother-infant bonding, reducing stress and improving neurocognitive outcome in low birth weight premature infants. [16]

The introduction of NIDCAP (Newborn Individualized Developmental Care and Assessment Program) has improved neurodevelopmental outcome in preterm infants with IUGR which was demonstrated in a randomized controlled trial. [17] Although Ohlsson et al.suggest in their recent metaanalysis that NIDCAP did improve long-term neurodevelopmental or short-term medical outcomes in the whole neonatal population. [18]

The improvement of continuous bedside monitoring for physiological and neurological variables plays an essential role in everyday neonatal practice. Readily avaiable imaging methods from bedside sonography to MRI examinations are part of the daily routine for caregivers of premature infants in level 4 perinatal centers.

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3.2. Brain development in the neonatal period

3.2.1. Structural Overview

In the last two trimesters an immense macro- and microstructural change takes place in the neonatal brain. To visualise the macrostructural changes, the process of cortical folding can be displayed with fetal MRI. The following figure depicts the development of the primary sensory and motor cortex. [19]

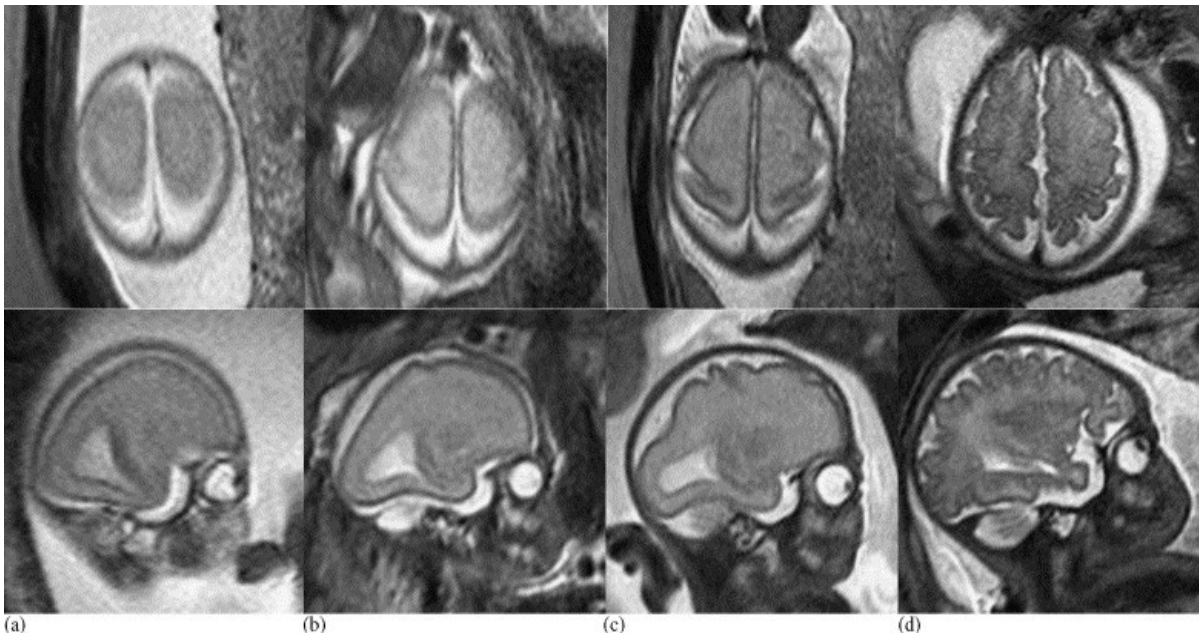

Figure 3.2.1. Fetal development of primary sensory and motor cortex. Axial (upper row) and parasagittal (lower row) T2-weighted sequences of fetuses at 20 GW (a), 25 GW (b), 27 GW (c), and 32 GW (d). Beginning with 25 GW, the central sulcus can be delineated as a slight indentation of the posterolateral aspects of both hemispheres.

The microstructural changes during the second and third trimesters include the formation of synaptic connections and axonal ingrowth leading to cortical folding and grey matter formation. Altough most cortical neurons have migrated in the first trimester, neurogenesis continues in the superior ventricular zone and subplate neurons are at their peak of activity. Oligodendroglia in the subplate plays a role in myelin formation and produce guidance molecules for migrating neural cells. Preoligodendrocyte cells are susceptable to hypoxic injury, leading to maturation arrest of this cell population and

consequent myelination failure with accompanying white matter injury. [20] Finally in the third trimester maturation of oligodendroglial lineage and the initiation of myelination is completed and glial and neural cells migrate into their final position in the cortex. [21]

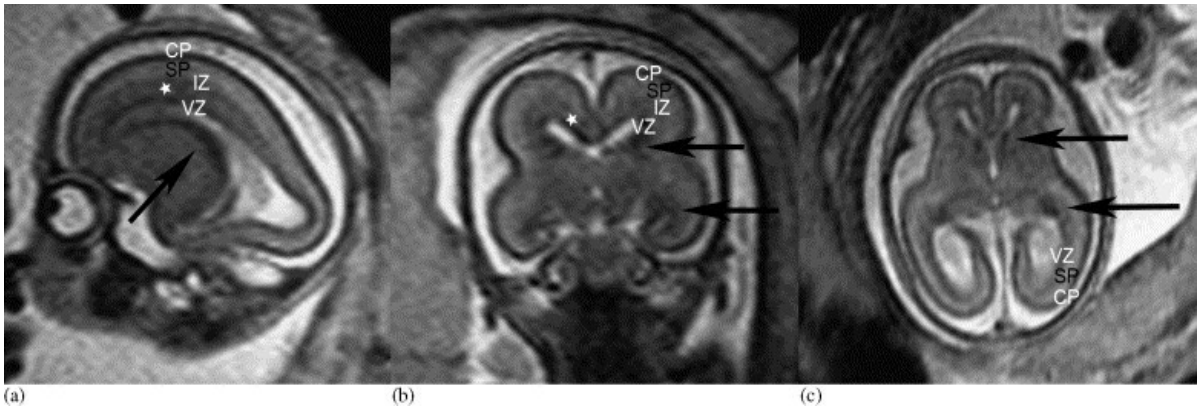

Figure 3.2.2. Sagittal (a), coronal (b), and axial (c) T2-weighted sections of a 20 GW fetus. The black arrows indicate the hypointense signal in the superior and inferior parts of the ganglionic eminence. The laminar organization of the fetal brain is visible—the hypointense cortical plate (CP), the hyperintense signal of the subplate (SP), the slim hypointense intermediate zone (IZ), and the hypointense ventricular zone (VZ). The subventricular zone can also be identified in the frontal regions (asterisks). [19]

3.2.2. Functional Overview and Plasticity

During the first trimester, sensory- and motor pathways fully develop in the fetus. Complex functions such as circadian rhytmus and sleep organisation, start to function in the second trimester although responsible structures such as thalamus and brainstem are already functioning previously. [22] Resting state MRI examinations provide data that these networks are not only present at the second trimester, but also show different maturational patterns in different brain regions. Infant fMRI studies have shown that neural networks cover greater anatomical distances later in development and that the most developed functional connectivity appears to exist in regions related to sensation and action. [23] Other studies have demonstrated that preterm children and adolescents have decreased cortical gray matter, cortical white matter, deep gray matter, cerebellar and total brain volumes when compared to age-matched term control subjects. [24-28] Diffusion tensor imaging studies have demonstrated microstructural changes in both the corpus

callosum and those intra-hemispheric association fibers subserving language skills. . [29] Interestingly in contrast to these data documenting aberrant brain development, behavioral studies suggest a pattern of recovery and preserved performance. [30]

Preterm children show a stronger neural circuit between Wernicke's area and alternative language regions in passive language tasks using fMRI when compared to term controls at 8 years of age, suggesting alternative brain development and plasticity of neural processing. [31]

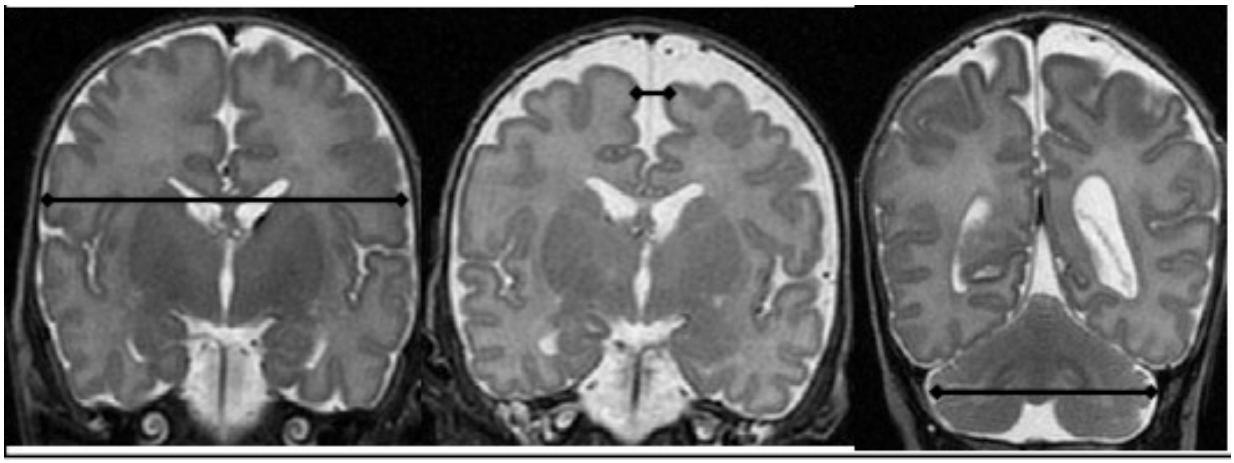

Figure 3.2.2.1. Premature Brain at Term. . Inder TE Pediatrics. 2005 Feb;115(2):286 As it is shown in the above figure, premature infants present smaller brain volumes, less cortical folding and narrower corpus callosum. [32]

The work of Staudt shows that sensorimotor reorganization with congenital hemiparesis is dependent on the time of injury during brain development. Ipsilateral corticospinal networks were further developed and the extent of motor dysfunction was smaller in case of early neonatal injury when compared to later injuries. This example demonstrates, that the efficacy of reorganization with ipsilateral corticospinal tracts indeed decreases during pregnancy. [33]

3.3. Neurophysiological methods in the neonatal period

Neonatal application of clinical neurophysiology has a steadily increasing role in the daily practice of neonatal intensive care units and follow up clinics. The importance of neurophysiological methods, mainly conventional EEG are recognised not only in epileptic syndromes and seizure diagnosis with treatment monitoring in intracranial pathologies and malformations, but also in the assessment of structural maturation and neural development.

In the assessment of the severity of neonatal encephalopathy and its prognosis conventional EEG and amplitude integrated EEG (aEEG) has proved to be an important an established tools for clinical decision makers.

Evoked potentials are electrical potential changes of sensory receptors, neural pathways and the brain following external or endogenous stimuli. Evoked potentials have attracted less interest in the neonatal population due to the lack of standardised maturational data in extreme premature infant and the difficulty of the measurement in the unstable patient treated mainly under intensive care. Nevertheless they offer precise answers to specific questions of neurological functions in the neonate. The following sensory evoked potentials are used occasionally: VEP-s in visual function analysis, AEP-s in auditory screening, SSEP in intraoperative monitoring. Event related potentials (ERP-s) like Miss Match Negativity (MMN) with P300 are used mainly in neuroscientific and cognitive developmental research. Brainstem auditory evoked potentials (BAEP) have shown a positive correlation with diffusion changes in MRI in the pons and impaired later neuromotor outcome. [34]

3.3.1. The role of neurophysiological methods

There is a growing need for continuous brain monitoring on the NICU, as adverse effects of everyday intensive therapy, infections, and immaturity present a risk for developing brain injuries and consequently influencing long term neurodevelopmental outcome.

Conventional EEG was the first method used successfully in the neonatal population. Due to increasing survival rates especially among the very premature population (<28 weeks of gestation) the prevention from later neurological deficit currently becomes even more important. Despite recent advances in perinatal care the incidence of impaired outcome in preterm infants has not decreased. Rates of cerebral palsy and overt cerebral lesions (cystic periventricular leukomalacia and peri/intraventricular hemorrhage) are decreasing, but the incidence of neurodevelopmental impairment remains high in preterm infants. This is explained by the understanding of different mechanisms in brain injury (for example inflammation, oxidative stress, impaired connectivity) and result in mainly cognitive impairment.[35, 36] A quantitative analysis of studies published between 1998 and 2008 shows that very preterm babies have moderate to

severe deficits in academic achievement, attention problems, internalizing behavioral problems and poor executive function compared to controls.[37] These issues are strongly associated with cognitive impairment. Therefore greater attention needs to be directed toward preterm neonatal populations to better understand brain adaptation both with and without medical complications. Neurophysiologic surveillance is necessary in these infants to adequately asses cerebral function and is difficult within this population by clinical aspects only. Conventional EEG is today´s gold standard for neurophysiologic diagnosis. Nevertheless it is not suitable for continuous recording since producing large data volumes which cannot be assessed directly at the bedside. In an effort to solve this problem, various methods of reducing and compressing the EEG signal have been developed, the amplitude-integrated EEG (aEEG), being one of them.

3.3.2. Conventional EEG

The electroencephalogram (EEGor conventional EEG) is a signal recorded from scalp electrodes and derived from the electrical activity of cortical neurons. The EEG signal represents the synchronious activity of neurons arranged at right angles to the surface, mainly the pyramidal neurons. The EEG changes through the neonatal period and childhood, thus it is essential to compare EEG measurements with normal values at the same maturational stage. EEG should be interpreted for background pattern, reaction to external stimuli, level of consciousness and for presenting pathological phenomena.

Conventional visual classification of the EEG signal of different brain regions has been the standard of analysis since the 1960s when first neonatal recordings were performed. Today more than 80% of extremely premature infants between 24-28 weeks of gestation survive. Therefore in the analysis of EEG signal there has been a growing need for more reliable automatic methods, being suitable for this specific population. There are several algorithms that have been developed in recent years, with different mathematical models to analyse not only the amplitude and frequency, but also the phase synchrony, coherence and temporal profiles of the EEG signal. [38, 39]

New nomenclature has emerged specifically for the premature population such as spontaneous activity transients (SATs), which constitute the most salient feature on EEG during the preterm period. This work has been based on animal models, showing that the characteristic discontinuous pattern found in premature infants is a universal phenomenon

during development in different species. These spontaneous bursts of activity, which are related to the excitatory role of GABAergic transmission during early development not only characterize the premature EEG, but also have been linked to the development of intracortical connections and neuronal wiring. SATs constitute of a very slow 0,1-0,5 Hz, with nesting activity at several higher frequencies. This activity represents the organization and development of thalamo-cortical connections, when neurons migrate from the subplate into the cortical plate in the primary sensory cortices. [40] SATs are shown to be useful in the everyday Neonatal Intensive Care Unit setting giving reliable information about the current clinical state and outcome of premature infants. [41] The development of sleep-wake-cycling is one of the ontogenetic oldest mechanisms of the developing brain and shows the integrity of a normal brain during development.

Pathological patterns should be differentiated as localised or generalised events of the recorded EEG. Seizure detection and localisation is the most common indication of neonatal EEGs, although research suggests that the electroclinical correlation of seizures are very poor in the neonatal population. Murray and Boyle suggest that only third of neonatal EEG seizures display clinical signs on video EEG recording and moreover only 60% of these are recognised at all. [42] They point out that unrecognised seizure burden is a serious problem, as untreated seizures can cause apoptosis of nerve cells and impaired neurogenesis suggested by animal research data.[43]

The seizures can be detected using conventional EEG in children presenting with IVH and the following hydrocephalus. [44] It is also usefull in case of subarachnoideal bleeding, metabolic diseases and most commonly in hypoxic ischaemic encephalopathy. [45]

3.3.3. Amplitude Integrated EEG

For early identification of infants at high risk and to optimize treatment, it is mandatory to have access to a reliable validated diagnostic method with excellent predictive value for later neurodevelopmental outcome. The aEEG is a readily available, informative and reliable technique for continuous non-invasive monitoring of brain activity even in extremely premature infants. Amplitude-integrated EEG is a simple method for continuous bedside monitoring of neurophysiological parameters in the neonatal intensive care unit setting. As our group has recently shown, aEEG has a predictive value for later

outcome in preterm infants and can therefore be used as an early prognostic tool for neurodevelopmental outcome. [46]

We have found emerging sleep-wake cycles as early as 24-25 weeks of gestation in neurologically healthy premature infants. [47] On the contrary premature infants with intraventricular hemorrhage exhibited a significant delay in emergence of their sleep-wake cycles, on average with 32 weeks of gestation. [48] We know that at this early age the development of intercellular connections of the brain and synaptic branching is still in development and that these processes take place mainly during sleep. [49] Consequently, the early emergence of sleep-wake cycles is the sign of normal brain development at this early age. On the other hand the earlier regular sleep-wake rhythm would lead to a better synaptic development and wiring resulting in even better later neuro-developmental outcome. Kostovic et al. shows, that neurocognitive outcome depends on the active connection of different brain circuits during prenatal and early postnatal life. The period between 24 to 28 weeks of gestation in extremely premature infants is a very important time, because not only maturation but also the migration of the neural cells are still going on. [46]

3.3.4. Visual Evoked Potentials

Visual Evoked potentials can be evoked by brief changes either in luminance (flash visual evoked potentials fVEP) or in the contrast (pattern VEP, pVEP) within the visual field. As there is no need for fixation to evoke the fVEP and luminance changes are detected through the closed eyelids as well, it is an optimal method to use in the non-cooperative neonatal population. VEP waveforms are labeled according to their polarity and the mean latency. Adult pVEP have three peaks N70, P100 and N145. fVEPs have I-VI. peaks, where the most reliable components are peak III (corresponding to N70) and peak IV (corresponding to P100). Maturational data is aviable for premature infants and children for both VEPs. P100 a positive Wave at 100ms emerges soon after birth.

VEPs assist in the diagnosis of varios optic nerve disorders, such as optic neuritis, optic nerve atrophy, hypoplasia, tumors and compression and play an important part in the diagnosis of multiple sclerosis. They permit early identification of disfunction and can be used to monitor progress. VEPs were found to be useful in the neonatal population with regard to prediction of neurodevelopmental outcome. [50-52]

.They seem particularly helpful in patients with PHVD as the visual pathway is adjacent to the lateral ventricles. [53] Therefore, an increase in ventricular width seems to lead to an early compromise of fVEP values.

VEP have shown to be of predictive value in detecting increasing intracranial pressure in children as VEP latencies increased with the increase of intracranial pressure and normalised after neurosurgical intervention. [54]

3.3.5. Event Related Potentials

Event related potential (ERP) is a response to a stimulus, where from many trials the results are averaged together, causing random brain activity to be averaged out and the relevant waveform to remain. Currently, ERP is one of the most widely used methods in cognitive neuroscience research to study the physiological correlates of sensory, perceptual and cognitive activity associated with processing information. Event-related potentials are caused by the "higher" processes of deeper brain structures or associative cortical areas, that might involve memory, expectation, attention, or changes in the mental state, while evoked potentials are resposes of cortical areas to sensory stimuli.

Mismatch negativity (MMN) is an event-related potential (ERP) component that provides a good measure of auditory perception and function and is typically observed between 100 and 250 ms. [55, 56] MMN is generated by the automatic response of the brain to a mismatch in auditory stimulation. It is elicited when a deviant stimulus (e.g., with a probability of 15%) appears within a train of repeatedly presented standard stimuli (e.g., with a high probability of 85%). The MMN is observed irrespective of the subject's direction of attention and is a good measure of the auditory system's ability to detect differences between sounds. [57] It is often used in experimental psychology in non-cooperative subjects, such as infants and young children..

3.4. Imaging of the neonatal brain

3.4.1. The role of imaging methods

Preterm infants are at high risk of developing brain injury. Neuroimaging does not only play an important role in prognosticating later neurological problems, but provides also essential information and support for the neonatologist in clinical decision-making in

critically ill neonates. MR imaging of the premature infant has been proved to be superior to the widely used serial ultrasonographic examinations. [58, 59] In one study MRI has enabled a non-invasive high resolution evaluation of the developing brain, where several studies have shown delayed grey-white matter differentiation, and diffuse white matter signalintensities after premature birth. [60] They also present a smaller corpus callosum, less mielinated white matter, larger ventricles, altogether smaller global and local brain volumes, when compared to healthy controls. [21]

Sonography is the method of choice for prenatal and postnatal malformation screening but it does not always provide sufficient information for correct diagnosis, adequate abnormality evaluation or consequent outcome information. Fetal magnetic resonance imaging (MRI) is considered as a valuable second line imaging tool after sonography for confirmation, completion and correction of regular ultrasound findings. [61] Fetal MRI has proven its value in the evaluation of central nervous system pathologies, especially of midline and posterior fossa malformations. Special sequences has been developed for this non-sedated, ever moving population. [19]

3.4.2. Magnetic Resonance Imaging

Specific sequences have been adapted for premature infants and newborns from research tools to routine imaging protocolls. Regular protocols including fast spin-echo T1/T2-weighted (w) sequences with long repetition-time and echo times in 3 section planes are part of routine protocolls of radiologists studying the neonatal brain and are useful mainly in the study of brain anatomy, malformations, bleeding and hydrocephalus.

Diffusion tensor imaging (DTI) enables the study of establishment of brain connectivity and plasticity. It is based on measures of water diffusion in biological tissues and is a powerfull tool to study white matter development and abnormalities. Fractional anisotropy (FA) can be quantified in different brain regions and show correlations with neurodevelopmental outcomes. FA values obtained in preterms at term-equivalent are lower in the right posterior limb of the internal capsule and at the splenum of the corpus callosum in case of developing cerebral palsy with two years of age. [62] Fibre tracking a voxel based analysis of DTI, enables the presentation of white matter tracts and their connectivity. A number of studies using DTI to visualize white matter tracts in neonates with white matter injuries and in older children with cerebral palsy have been published

with promising results. One study of 24 infants with birthweight below 1500 g who had DTI at 37 weeks postmenstrual age found a strong correlation between low fractional anisotropy values in the posterior limbs of the internal capsule and both diagnosis of cerebral palsy and severity of gait problems on outcome evaluations at 4 years. [63]

Functional MRI (fMRI) is a novel method in newborns, but is a promising research tool in neural processing and resting state connectivity. It refers to regional changes in signals that correlate with brain functional activity. It uses deoxygenated haemoglobin levels or otherwise known as BOLD (blood oxygenation level dependent) signals, which indirectly depicts regional activity.[64] Neonatal Current research concentrates on the development of neonatal brain networks with the use of resting state activity in order to understand maturation in a normaly developing fetus. [65]

Proton magnetic resonance spectroscopy of the brain is a non-invasive technique that supplies information about the presence and levels of metabolites, such as N-acetylaspartate (NAA), choline (Cho), creatinine (Cr) and other substances. They provide usefull information in metabolic diseases, neurodegenerative disorders and in neonatal encephalopathy as well.[66]

3.4.3. Cranial Ultrasound

Serial head ultrasounds are a valuable bedside tool for following brain development and and occuring intracranial pathologi in even the sickest preterm infants. Two studies have demonstrated that many preterm infants have a reduction in the size of the corpus callosum at term (compared with term controls); this is associated with lower gestational age at birth and with cerebral palsy and lower cognitive scores [67]

Blood velocities of cerebral arteries are especially usefull in hypoxic ischemic encephalopathy. Serial cranial sonographic examinations are part of the daily routine on the neonatal ward for the detection of common brain pathologies such as intracranial bleedings, PVL, PHVD and neonatal encephalopathy. IVH is classified according to Papile [68] PHVD is classified according to the ventricular index of Levene and recently an additional anterior horn width (AHW) and thalamo-occipital distance (TOD) helps better evaluation [69] Neonatal encephalopathy is classified according to the classification of Deeg et al.

Sonography has its limitations as well. De Vries et al. showed that the sensitivity of sequential ultrasound imaging for detecting abnormalities to predict later cerebral palsy was 76%, while Miller et al. described, that, although the positive predictive value of acute white matter injury was high, the sensitivity of these findings were low among premature infants.[70, 71] Further analysis with MRI is needed to clarify the extent of neuropathology found on ultrasound examinations.

3.4.4. Computer Tomography

Computer Tomography (CT) has a very limited role in neonatal brain imaging. As it is a major radiation burden for the developing brain, it is only used in emergency situations, when MR imaging would take too long to organize. Neonatal cranial CT examinations are still in use in case of subarachnoideal or subdural bleeding with progressive brain oedema or head trauma with skull injury and need for immediate neurosurgical intervention.

3.5. Common morbidities of the central nervous system during the neonatal period

3.5.1. Intraventricular haemorrhage

Intraventricular haemorrhage (IVH) is still the most common reason for brain injury in preterm infants. Additional progressive posthaemorrhagic ventricular dilatation (PHVD) is known to be associated with subsequent white matter damage and therefore increases the risk for neurodevelopmental disability furthermore. [72]

It is most commonly found in the preterm infants and the incidence is between 15-20% under 32 weeks of gestation. In the rare cases in term infants IVH is related to birth trauma and originate from the choroid plexus, while in preterms the fragile involuting vessels of the subependymal germinal matrix and their rupture are responsible for the bleeding. Sudden changes in cerebral venous pressure, due to hypervolemia, hypoglycaemia, pneumothorax or seizures also contribute to IVH. [73] The following cranial ultrasound images of figure 3.5.1. show the different grades of IVH.

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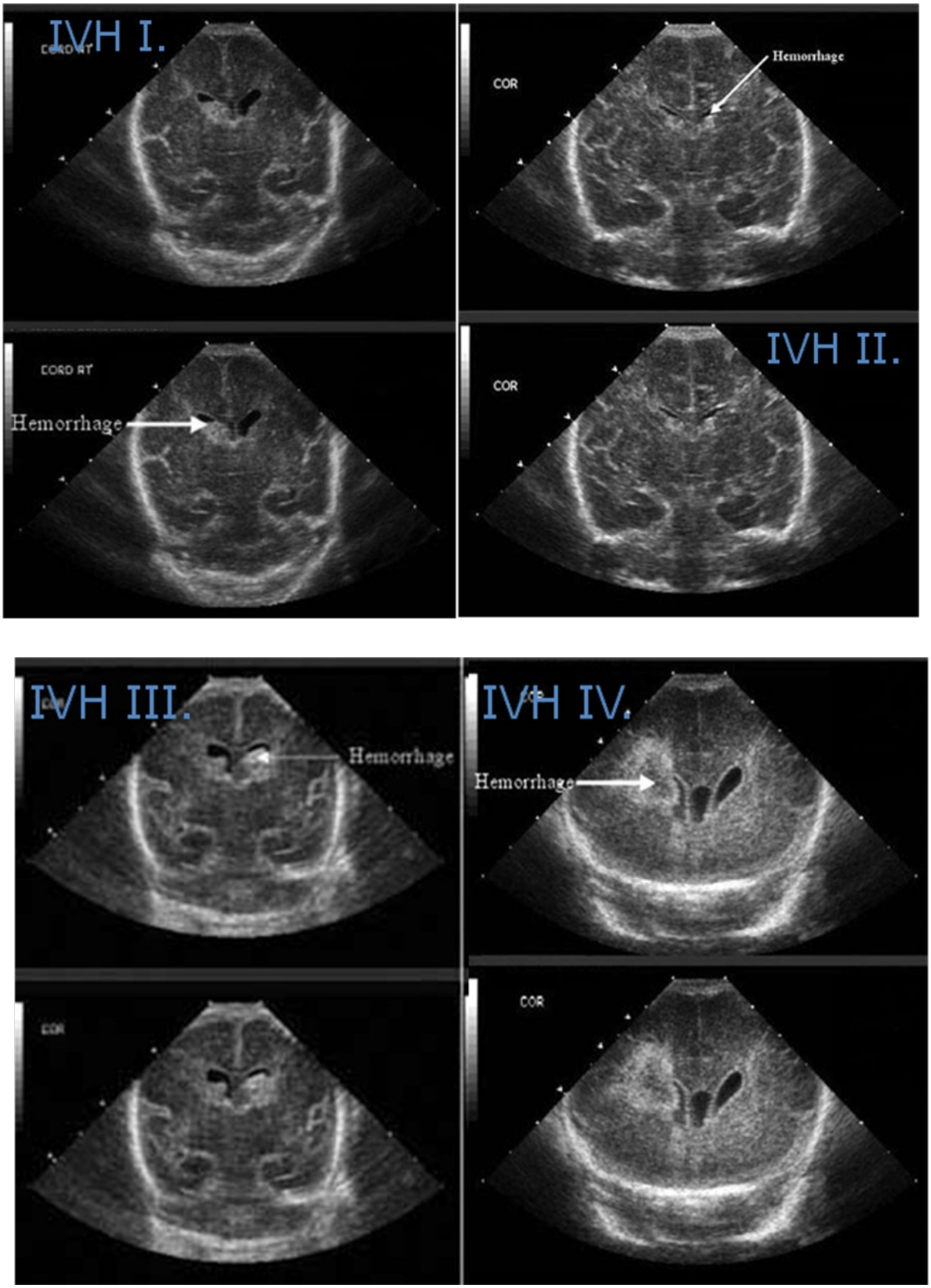

Figure 3.5.1.Cranial ultrasound images of different grades of IVH. White arrows show the heamorrhage at the pictures. IVH I-IV is presented from own patient population.

3.5.2. Posthemmorhagic ventricular dilatation

Hydrocephalus after intraventricular hemorrhage is as a major complication of preterm birth and is particulalry problematic to treat. The pathophysiology of hydrocephalus is usually ascribed to fibrosing arachnoiditis, meningeal fibrosis and subependymal gliosis, which impair flow and resorption of cerebrospinal fluid (CSF). IVH is associated with damage to periventricular white matter and the damage is exacerbated by the development of hydrocephalus; combinations of pressure, distortion, ischaemia, inflammation, and free radical-mediated injury are probably responsible. The damage to white matter accounts for the high frequency of cerebral palsy in this group of infants. 25% of infants with IVH develop a progressive hydrocephalus and the incidence increases with severity. Clinical signs of hypertension are not always reliable defining severity of PHVD, imaging and neurophysiological examinations help to determine management and prognosis.

3.5.3. Asphyxia

Perinatal asphyxia is a condition of the neonatal brain due to hypoxemia and hypercarbia, due to impaired gas exchange. Characteristicly infants present with abnormal neurobehavior (defined by Sarnat and Sarnat), seizures, impaired vertebral blood flow. Additional complications of neonatal encephalopathy include a multiorgan dysfunction with mostly renal, cardial, pulmonary and hepatic abnormalities. Despite joint efforts of neonatologists and obstetritians the incidence has been stable between 0,5-2%, with 10-20% death and 25-50% neurodevelopmental disability in the surviving population. Additional hematologic problems require a complex intensive management of these patients. Cranial ultrasound is an essential tool of bedside monitoring, but MRI has proved superior defining the extent of white matter abnormalities, restricted diffusion reflecting cytotoxic edema, or spectroscopy measuring metabolite ratios, such as Cho/Crea or NAA/Crea or lactate levels. aEEG has been proved to be a good outcome predictor when carried out within 48 hours after birth. [74]

3.5.4. Periventricular leukomalacia and diffuse white matter injury

Periventricular leukomalacia is a common injury of the preterm population with necrosis, gliosis and disruption of axons at border zones of vascular supply. Hypoxia-ischeamia is followed by cystic necrosis and diffuse white matter abnormalities with

hypertrophic astrocytes and loss of oligodendrocytes resulting in overall decrease of cerebral volume. The incidence of large cystic PVL decreases, but diffuse white matter injury remains significant, resulting in 10% CP and 50% mild disabilities. [75]

3.6. Neurodevelopmental Outcomes

Preterm children compared to term controls present with a variety of neurodevelopmental problems. At 6 years of age 30-40% have minor developmental impairment and 20% have major disabilities. Among these major disabilities 42–47% of children had cerebral palsy, while 27%, 37% and 23% of these children had a significant cognitive, visual and hearing impairments, respectively. [76] An increase in cerebral palsy with decreasing birthweight and gestational age category is a consistent finding in preterm outcome studies. This finding is not limited to extremely preterm infants. [75] EPIPAGE study reports an increase in cerebral palsy with each preterm week: 0.7%, 3.7%, 4.1%, 8.7% and 6.3% in children with gestational age 34, 33, 32, 31 and 30 weeks, respectively ($P < 0.01$).The intellectual deficit of preterm children present also in adolescent and young adulthood. [77] The study from Walther also shows that mortality and major handicap are relatively stable over time during follow up, but minor disabilities and developmental problems show a substantial increase over time.

Neurodevelopmental outcome measuremets have traditionally several subgroups, such as motor- and cognitiv outcomes, sensory impairment, -and language skills. Although many preterm infants demonstrate neuromotor abnormalities on examination, most do not develop cerebral palsy, but mild persistent neuromotor abnormalities (e.g. asymmetries, tight heel cords), motor planning problems and/or sensorimotor integration problems that lead to functional impairments (e.g. tying shoelaces), academic difficulties (e.g. writing). [76]

Recent studies have not only confirmed that children born preterm have more cognitive impairments and academic difficulties than fullterm controls, but they also suggest that these are more common than motor, visual or hearing impairments, as they apper up to 55% of premature infants under 27th week of gestation. [78]

Difficulties with reading and spelling increased with decreasing gestational age (and birthweights) in a Danish study of 11–13 year olds. [79] They found significant differences between children born at 33–36 weeks (and at 37–38 weeks) compared with

children born at 39–40 weeks gestation, which points out the importance of risk factors for the late preterm population as well.

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4. Objectives

The author of this paper would like to present through her research different aspects of neonatal neurophysiological examinations and neuroimaging methods, in order to better understand the development of neonatal brain pathologies and their effect on neurodevelopmental outcome.

4.1. Feasability of Functional Neurophysiological Methods and optimalization of neonatal care

4.1.1. Hydrocephalus Study

The aims of the present prospective study were firstly to evaluate the role of VEPs and aEEG in the monitoring of elevated intracranial pressure in congenital hydrocephalus and the development of PHVD in preterm infants and to define pattern changes with decompressing neurosurgical interventions. The second aim was to correlate our findings with the degree of ventricular dilatation and Doppler sonography. Thirdly, we wanted to compare late versus early intervention with the primary outcome, the need for long term ventriculoperitoneal shunt insertion. Our hypothesis was that both neurophysiological methods show changes in case of intracranial pressure elevation or normalisation.

4.2. Optimalisation of Cerebral Imaging methods

4.2.1. MRI-compatible incubator Study

Aim of our study was to assess our initial experience with an MRI-compatible incubator and analyse its impact on examination feasibility and further clinical management. In order to acheav this we first analyzed the use of MRI itself in unstable patients under intensive care management and secondly the usefulness of a special device (MRI-compatible incubator) making MRI examination in unstable patients more feasible. Because the main advantage of the MRI-compatible incubator is optimizing thermoregulation during the MRI, we separately analyzed the usefulness in patients weighing under 2000g.

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4.3. Use of functional neurophysiological or imaging methods and prediction of outcome

4.3.1. Asphyxia Study

The aim of our retrospective study was to analyse early versus late MRI and aEEG data separately and correlate its effect on the prognostic outcome of children with asphyxia at two years of age. Secondary aim was to combine aEEG and MRI data in order to develop a more exact prognostic value for this patient population. Our hypothesis was that neurodevelopmetal outcomes are predictable safely with the use of aEEG and there will be a difference between late versus eraly measurements. The second hypothesis regarding MRI was that it has a stable predicitve value independent of the timing of the measurement.

4.3.2. Mismatch Negativity Study

The aim of the Mismatch-Negativity (MMN) study was to test the maturation of phoneme and stress discrimination in case of natural speech in premature infants and healthy controls at 6 and 10 months of age. The main principle along which we planned our experiment is to investigate the typical stress information at the word level. Hence we used a complex pattern of acoustic cues while varying stress information using the miss match negativity paradigm of event related potentials. Our hypothesis was that we will find differences between the two age groups in case of both phoneme and stress detection, but preterm infants will show maturational lag only in case of stress processing.

4.3.3. Intraventricular Haemorrhage Study

The aim of this prospective study was to compare outcomes of preterm infants with different grades of IVH born below 32 weeks of gestational age (GA) with outcome of controls without IVH. Emphasis was on the comparison of the influence of low grade IVH on the neurodevelopmental outcome. Cranial ultrasound examinations were carried out on the 1st 3rd, 5th, 7th, and 10th day of life and then once a week until discharge. Our first hypothesis was that gestational age has an effect on neurodeveopmental outcome in case of

IVH. The second hypothesis was, that neurodevelopmental outcome correlates with the severity of IVH in preterm infants.

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5. Methods

5.1. Hydrocephalus Study

Patients who developed a posthaemorrhagic hydrocephalus and also required neurosurgical intervention were eligible for inclusion. Subjects with IVH received serial CUS scans every second day. PHVD was defined as the progressive increase of ventricular width following IVH as seen on CUS. In the case of PHVD, VEPs and aEEG examinations were performed at least once weekly before and after neurosurgical intervention in order to follow both the development of ventricular dilatation and the reduction of ventricular width after insertion of an external ventricluar drain (EVD) or implantation of a ventriculo-peritoneal shunt (VP-shunt). During the study period 17 patients met the inclusion criteria. In all cases we were able to perform fVEPs and aEEGs prior to and after placement of CSF drainage systems.

In a further analysis the PHVD study population was compared to patients with congenital hydrocephalus. An additional 3 patients were included, and followed similarly with VEPs, aEEG and CUS until neurosurgical intervention. The underlying pathologies resulting in congenital hydrocephalus were, 1.rhombencephalosynapsis, 2.teratoid tumor of the neck compressing the fourth ventricle and 3.subcortical heterotropies with partial corpus callosum agenesis and aqueductal stenosis.

Cerebral ultrasound scans were performed on days 1, 3, 5, 7, and 10 of life and then once a week until discharge by using an Acuson 128XP (Mountainview, CA) with a 7.5-MHz transducer. IVH and periventricular leukomalacia were classified according to Papile and de Vries et al, respectively. [68, 71] PHVD was classified according to the criteria of the ventricular index to Levene (Ventricular width was measured in the coronal plane from the lateral wall of the body of the lateral ventricle to the falx), and a neurosurgical intervention of external ventricular drainage was latest performed if the ventricle was wider than 4mm above the 97th percentile. [80] Additionally, anterior horn width (AHW) and thalamo-occipital distance (TOD) (according to reference values by Brouwer et al.) were evaluated.[81] [69] Using Doppler sonography, blood flow velocities and the resistance index were measured in the anterior cerebral artery. [82]

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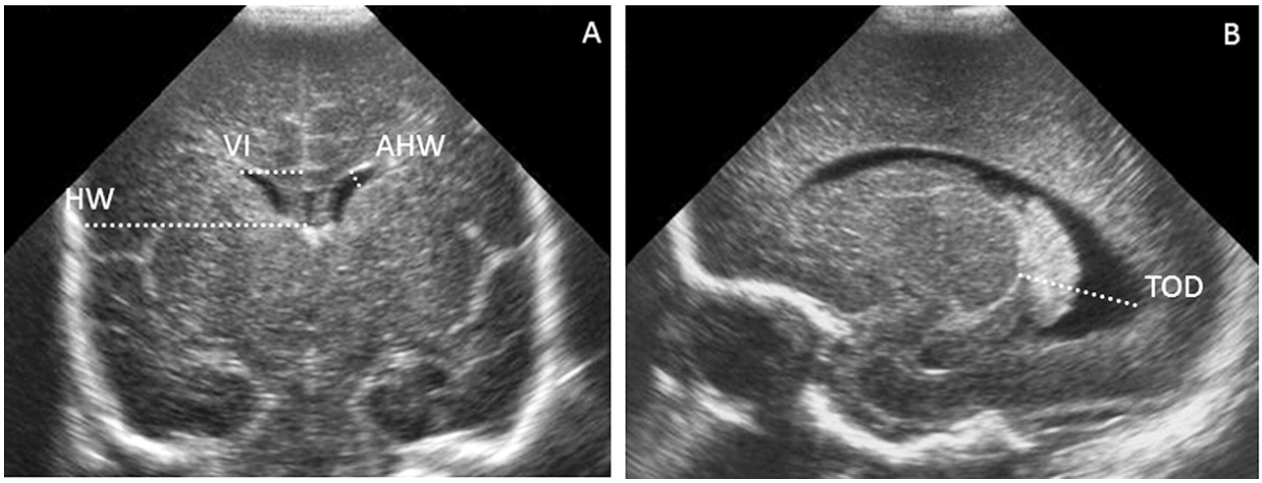

Figure 5.1.2.1. A) Measurements of the anterior horn width (AHW), the maximal diagonal width of the anterior horn, the ventricular index (VI), the distance between the falx and the lateral wall of the anterior horn and the frontal horn ratio, the ratio between the VI and corresponding hemispheric width, in the coronal plane at the level where the AHW appears maximal. (B) Measurement of the thalamo-occipital distance.

Congenital hydrocephalus was followed similarly and ventricular index, TOD and AHW was calculated. In case of these 3 patients an additional MRI has also taken place to define the extent of intracortical malformations.

Flash visual evoked potentials fVEP measurements were performed weekly in infants with developing PHVD and congenital ventricular dilatation. As soon as the ventricular index reached the 97th percentile recordings were performed twice weekly until neurosurgical intervention was performed. fVEPs were recorded using the Neuropack 8 (Nihon Kohden). The fVEP measurements were done in closed cots or open-air units, both covered with a blanket in order to create a semidark environment. The stimulating source was a red light emitting diode goggle held at a distance of 5 cm in front of the infant's eyes. The evoked potentials were recorded using three cortical electrodes placed on the infants scalp (active electrodes at Oz and Fz, ground electrode at Cz according to the international 10/20-system). The stimulation frequency was 0.5 Hz, the electrical impedance below 5 kOhm and the emitted light energy was 0.4 Lux. Two courses aiming for 30 and 50 responses were averaged using a band pass filter of 1–100 Hz and a sweep time of 1 sec. Responses including excessive artefacts were automatically rejected and trials were performed together on both eyes (binocular). fVEP measurements were recorded during active sleep, determined using the simultaneously recorded aEEG

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background pattern and the assessment of the behavioural state of the infant. [83] Waveforms and latencies were then analysed off line for every measurement. Reproducible positive and negative waves were named according to the order of their appearance, N0, N1, P1, N2, P2 and N3 and were compared with the reference values published by Pike et al. [84]

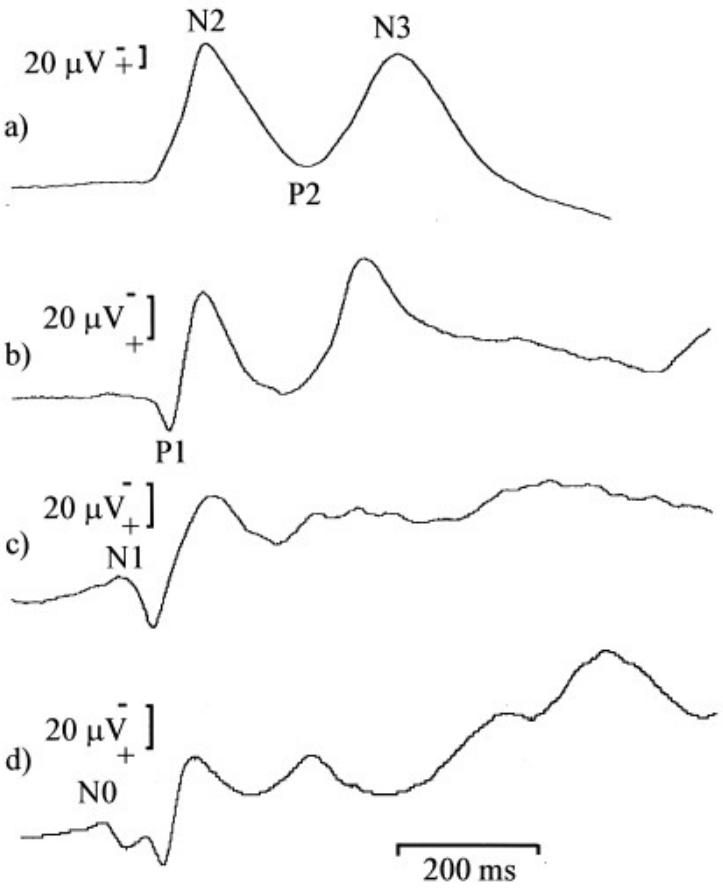

Fig. 1. Visual evoked responses recorded from infants of: (a) 27 weeks post-conceptional age, (b) 32 weeks post-conceptional age, (c) 35 weeks post-conceptional age, (d) 40 weeks post-conceptional age.

Figure 5.1.2.2. The evolution of common fVEP waveform during maturation. Results from Pike et al.

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Figure 5.1.2.3. The light emitting diode goggle is held in front of the eyes of the sleeping infant for binocular stimulation. Photo of a participant in our hydrocephalus study.

Amplitude-integrated EEG At the same time as fVEP measurements were performed, aEEG was recorded as a single-channel EEG from biparietal surface disk electrodes using a cerebral function monitor (Olympic Cerebral Function Monitor 6000). In brief, the obtained signal is filtered, rectified, smoothed and amplitude-integrated before it is written out at slow speed (6 cm/h) at the bedside. [85] Tracings were evaluated visually and classified according to the method previously described by Hellström-Westas et al. [86]

Descriptive analysis of the background activity of the aEEG tracings was done by dividing each trace in 10 min epochs and by calculating percentages of occurrence of the different patterns. Appearance of sleep-wake cycling (SWC) and seizure activity was noted within the entire recording. aEEG pattern was then scored according to the following:

1. Background activity (age-adequate distribution of pattern according to reference values previously published) [46, 69, 87]; a value within 25th and 75th percentiles for

every pattern was classified as ‘age-adequate’[69] 2. Appearance of SWC. 3. Presence or absence of seizure activity.

‘Normal aEEG-pattern’ (=score 0) was defined when all three categories were classified as normal, ‘moderately abnormal aEEG-pattern’ (=score 1) was defined when 1/3 categories were classified as abnormal and ‘severely abnormal aEEG-pattern’ (=score 2) was defined when 2 to 3/3 categories were classified as abnormal.

Conventional EEG with video was performed when seizure pattern was seen on aEEG. The aim of the EEG examination was to locate epileptogenic activity more precisely and to define seizure chracteristics, such as duration, generalisation, waveforms and electroclinical correlates. The System 98 software from Micromed was used, with using the neonatal version of 10/20 system with 8 electrodes for premature infants and 20 electrodes for term infants.

5.2. MRI-compatible Incubator Study

In a retrospective study we analysed the clinical and imaging data of neonates undergoing MR Imaging between 2003-2007. Our study population included all 129 premature and newborn infants during two consecutive time periods, undergoing MRI examinations at the Medical University Vienna, Austria. The first 18 months study period was between June 2003 and January 2005 and the second 18 months period with the MRI-compatible Incubator (INC) was between June 2005 and January 2007. The criteria of critically ill infants included one or more of the following: need for ventilation, the first day of life, unstable infant with bradycardias, desaturation and unstable blood pressure. Subanalysis of data of infants with a weight below 2000g during MRI examination is given separately.

The premature infants were placed into the INC on the NICU (approximately 10-20 min before examination was scheduled), then transportation to the radiology department of our clinic, which is located in another building. Transport time is approximately 10-15 min and although all MRI examinations are previously arranged, waiting times vary between 0 and maximal 30 minutes. In the first time period the transport incubator was used with similar transport and waiting times, but in this case an extra 10-30 minutes were added to the whole process, while the infants had to be stabilized in the MRI when repositioned.

The criteria for change in management after MRI examination was: starting, or finishing a medical therapy due to the result (anticonvulsive drugs, metabolic supplementation or special diet, antithrombotic or antibiotic drugs), or changes in clinical practice after imaging, such as initiation of surgical intervention or postoperative decision-making.

The criteria for change in the ultrasound diagnosis was unconfirmed diagnosis or additional diagnostic information provided by the MRI examination. Infants needing ventilation were hand ventilated with an Ambu-balloon during the MRI examination in the first period, while in the second period the Pneupac babyPAC 1000 ventilator was used. For monitoring the Invivo Precess (MeMed-Menges) was used in the first and the Invivo 4500 MRI Monitor in the second period. Both monitor and ventilator are integrated into the INC.

The same Philips Intera 1,5 Tesla (Philips-Best-The Netherlands) MRI System was available in both periods for the imaging of premature infants. Before the availability of the INC we used a birdcage knee coil; with the INC an in-built incubator head coil was used. For both coils the same sequences were used. Routine protocols including fast spin-echo T2-weighted (w) sequences with long repetition-time and echo times in 3 section planes, axial T1w spin-echo sequences, sagittal T1-w 3DGradient-echo sequences, diffusion w sequences. (standard newborn T2-weighted TSE sequence with TR 300ms, TE 140ms, duration 1.21 min., FOV 120, slice thickness 3mm, = gap maximum slices 28, Matrix 108x108, acquired voxel size 0.69/0.81/3mm, reconstructed voxel size= 0.43/0.4373mm). Angiography and spectroscopy were added in some cases. Diffusion-tensor imaging was done in 50% of the babies examined in the INC. Sequences, adapted from fetal protocols were done in cases of severe instability.[7] Imaging time was calculated from the beginning of the first sequence until the end of the last sequence.

MRI-Compatible Incubator

The MRI-Compatible Incubator from LMT MR Diagnostic Incubator Nomag IC 1.5 has been designed to provide a safe environment for the critically ill and very low weight premature infants with their special needs. (Figure 5.2.2.) The temperature and humidity regulators, the MRI compatible monitors and the ventilation support system are all necessary for the stability during transport and imaging of this patient group. The built

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in head coils and auditory shielding have improved the imaging process for both these small patients and the radiologist. The advantage of the in-built head coil is that its surrounds the infants head completely, leading to a better signal in the parietal regions of the brain.

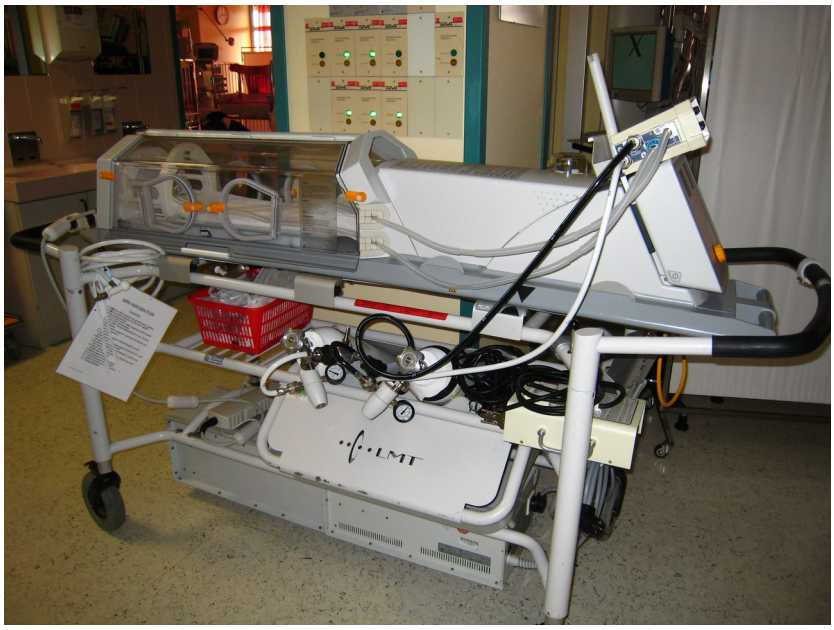

Figure 5.2.2. MRI Compatible Incubator used in our study. Ventillator and built in head coil are important part of the system.

5.3. Asphyxia Study

In a retrospective analysis we selected premature and term infants who developped a hypoxic ischeamic encephalopathie (HIE) between 2003 and 2006 and were admitted to the Neonatal Intensive Care Unit (NICU) at the Medical University of Vienna. Inclusion criteria included all infants with asphyxie, defined as 1) Apgar Score below 5 at one minute or 7 at 5 minutes, 2) cord pH below 7,0, Exclusion criteria were metabolic disorders, congenital malformations and genetic abnormalities. 142 participants met this criteria during the 4 year period. There was no hypothermia treatment avaiable at this time point on our neonatal ward. Neurodevelopmental outcome data at two years of age was collected at our follow up clinic. Further selection included only those patients who also underwent an MRI examination in the perinatal period (within 6 weeks after birth). Altogether 44 patients met this criteria.

Clinical and epidemiological data was collected. HIE was classified according to the clinical –neurological status by Sarnat. The severity of Sarnat stadiums range from light, to moderate, and severe. (I-III.) [88]

I. Mild HIE – Sarnat Stage I:Hyper-alert,Eyes wide open,Does not sleep,Irritable,No seizures Usually lasts ≤ 24 hours

II.Moderate HIE – Sarnat Stage II: Lethargy (difficult to rouse), Reduced tone of the extremities and/or trunk, Diminished brainstem reflexes (pupil/gag/suck), Possible clinical seizures

III.Severe HIE – Sarnat Stage III: Coma (cannot be roused), Weak or absent respiratory drive, No response to stimuli (may have spinal reflex to painful stimuli), Flaccid tone of the extremities and trunk (floppy), Diminished or absent brainstem reflexes (pupil/gag/suck) Diminished tendon reflexes EEG severely abnormal (suppressed or flat EEG with or without seizures)

Neurophysiological examination

Routine neurophysiological monitoring included continuous aEEG data collection within 6 hours of birth until the third day of life, after this period routine aEEG examinations were on weekly basis, unless clinical status indicated otherwise. Epileptic activity on aEEG or clinical signs of seizures were followed through with a conventional EEG with video for further analysis. The aEEG was recorded as a single-channel EEG from biparietal surface disk electrodes using a cerebral function monitor (Olympic CFM 6000). In brief, the obtained signal is filtered, rectified, smoothed and amplitude-integrated before it is written out at slow speed (6 cm/h) at the bedside. [87] The appearance of sleep-wake cycling, the occurrence of seizure activity and the distribution of background pattern was analyzed according to the previously published protocol by Klebermass. [46] Tracings were evaluated visually and classified according to the method previously described by Hellström-Westas et al. [86] Tracing were classified as 1. normal, 2. light, 3. moderately or 4. severely abnormal. The presence or absence of seizure activity was additionaly evaluated.

Magnetic resonance imaging

The Philips Intera 1,5 Tesla (Philips-Best-The Netherlands) MRI System was used. Routine protocols including fast spin-echo T2-weighted (w) sequences with long

repetition-time and echo times in 3 section planes, axial T1w spin-echo sequences, sagittal T1-w 3Dgradient-echo sequences, diffusion w sequences. (standard newborn T2-weighted TSE sequence with TR 300ms, TE 140ms, duration 1.21 min., FOV 120, slice thickness 3mm, = gap maximum slices 28, Matrix 108x108, acquired voxel size 0.69/0.81/3mm, reconstructed voxel size= 0.43/0.4373mm) were carried out. Diffusion tensor imaging was added in all but one case and spectroscopy were added in some cases. Sequences, adapted from fetal protocols were done in cases of severe instability[19] We grouped the time of the MRI Scans as early scans, within the first week of postnatal life and late scan between 1-6 weeks of postnatal life. We used the scoring system from Barkovich et al to identify changes in signal intensity of different regions. Regions of interest were basal ganglia/thalamus, posterior limb of the internal capsule, cortex and white matter were analysed (PLIC). [89] The maximum score was 5 when in all regions and the additional diffusion abnormalities were present, while 0 score represented a normal MRI.

Neurodevelopmental outcome

Outcome was assessed at two years of age using the Gross Motor Classification System and the Bayley Scales Psychomotor and Mental Developmental Index. The Bayley Scales of infant development were classified as normal when psychomotor (PDI) and mental developmental index (MDI) scores were >85 (± 1 SD of reference values). (Bayley N (1993) Bayley Scales of Infant Development II. Psychological Corp, San Antonio. Scheffzek scores were calculated according to neuroclinical status, in case of missing BS. [90]

5.4. Mismatch Negativity-Study

In a prospective study to analyse language development in the first year of life in term and preterm subjects we recruited eighty-nine infants to participate in the experiment. Fifty-two of them were excluded either because they did not match the strict selection criteria concerning age, birth weight, and gestational age (GA) characteristics, aiming to promote group homogeneity (n=12), or because of a low percentage of the artifact-free trials in the electrophysiological data due to infants crying, and/or frequent and excessive head and body movements(n=40) All preterm infants were recruited by using the database of the Follow up Center for Developmental Neurology, I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary. Normal cerebral ultrasound and hearing (oto-acoustic emission test) was the inclusion criteria. According to the ethical

requirements set by the Ethical Board responsible for permission we conducted the experiment at the clinic applying a portable EEG/ERP recording system (BrainAmp from BrainProductsGmbh.) after having the parents' written informed consent. The full-term infants were selected with help of pediatricians from the Health Care Centre, Vezérutca, Budapest, Hungary. The circumstances and conditions of the EEG recordings were similar to those of preterm infants.

We created 4 groups of infants based on age, and the term of birth. We had two age groups, 6-month-old infants with 19 participants and one of 10-month-old infants with 18 participants. Criteria used as preterm birth were the GA of weeks≤ 37 and/or birth weight≤ 1500 gram. Consequently we had 21 preterm and 16 full term subjects. During the experiment babies sat on their parents lap, in a silent room. We used different toys as distractor stimuli in order to prevent the babies to pay attention to the acoustic stimuli. The experimental stimuli were presented via two loudspeakers placed in equal distance (40 cm) from the infants'head on the left and on the right side.

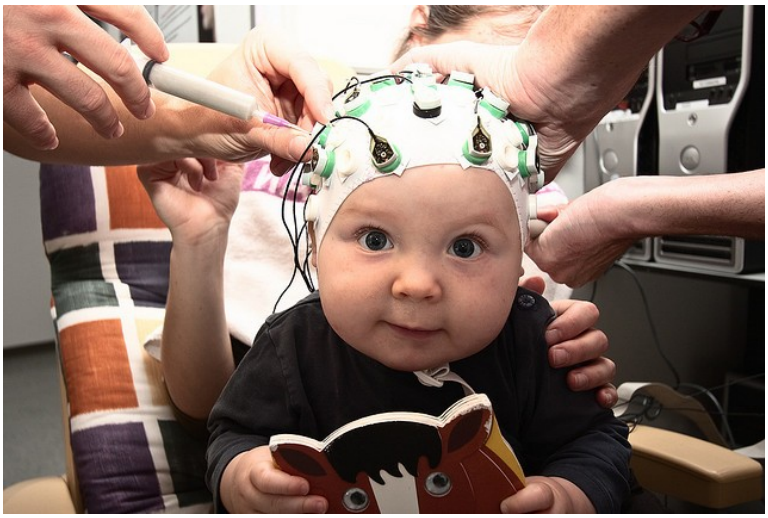

Figure 5.4.1. Young patients with an EEG cap before the experimental condition on the lap of his mother.

Stimuli and experimental conditions

We used a passive oddball paradigm with a standard Hungarian word `banán` (`banana` in English) and two deviants: a voiceless phoneme deviant (`panán`, which is meaningless in Hungarian), and a stress deviant where the stress was on the second syllable, instead of the first which is a normal stress pattern in Hungarian (`ban:án`) (for all details see. [91] The experimental stimuli consisted of Hungarian words uttered by a native

female speaker and recorded and digitized by means of a personal computer with a sampling rate of 44100 Hz. The stimuli were presented in random order; the probability of the deviant stimuli was 25%, and the stimulus onset asymmetry (SOA) varied randomly between 730 and 830 ms.The two deviants were presented in separate series (150 standard and 50 deviant stimuli; the order of two series was counterbalanced)

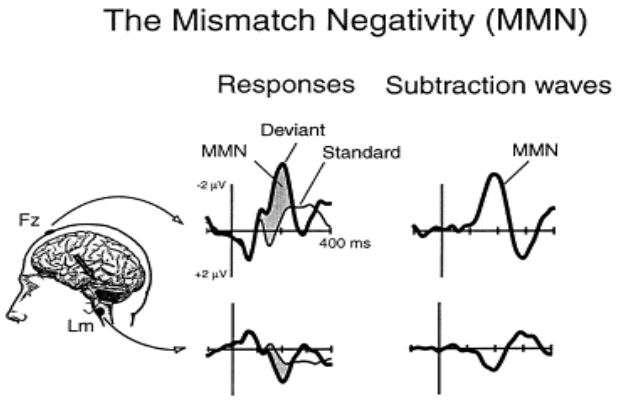

Figure. 5.4.2. Derivation of MMN response. The evoked responses to the standard and deviant stimuli are extracted and the result is depicted as a negative curve called the MMN at two different electrode positions.

Data collection and measurement

The electroencephalogram was recorded from 16 scalp locations: F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, Oz, O2, T3, T4, M1, M2. The reference electrode was at point Fpz, and the ground was between Fz and Fpzon the midline. The offline data analysis was performed by using the BrainVision Analyzer software (BrainProductsGmbh.). The original EEG was algebraically re-referenced to the average activity of all electrodes. Aband-pass filter of 0.3- 20Hz, 24 dB/octave was used.The raw EEG data was segmented into epochs of 800 ms, time-locked to the onset of the stimulus (-100 ms before onset to 700 ms after onset). Next, we applied an automatic artifact rejection method whereamplitudes above ±120μV were rejected. Participants whose recordings were below 20 artifact-free epochs were excluded from further analysis (see above).Then the segmented data was base-line corrected from -100 ms to the onset of the stimuli and finally, the remaining epochs were averaged.

5.5. IVH Study

The purpose of this prospective study was to compare outcome data of preterm infants with different grades of IVH with those of preterm infants without IVH in association to gestational age over a time period of twelve years. The main point of interest was the effect of low-grade IVH on neurodevelopmental outcome in this preterm cohort.

We included preterm infants with a gestational age below 32 weeks, who were admitted to the neonatal intensive care unit (Medical University of Vienna, Austria) between 1994 and 2005. Infants with additional periventricular leucomalacia and cerebellar lesions were excluded from the analysis. Neurodevelopmental assesment was carried out in our outpatient clinic. Demographic data include GA, birth weight, sex, antenatal steroids, multiple birth, mode of delivery and incidence of respiratory distress syndrome; chronic lung disease (defined as supplementary oxygen at corrected age of 36 weeks of gestation); patent ductus arteriosus (PDA) (defined as PDA needing treatment); necrotising enterocolitis (NEC) (defined as any NEC≥Bell's stage 1); amniotic infection syndrome or chorioamnionitis (defined as clinical and or histological signs of chorioamnionitis); posthemorrhagic hydrocephalus (defined as any ventricular dilatation after IVH); Shunt—necessity of implantation of ventriculoperitoneal shunt and retinopathy of prematurity (ROP) (defined as appearence of ROP of any grade). GA was determined from the date of the mother's last menstrual period and according to antenatal ultrasound scans. For further statistical analysis, the study group was divided in two age groups (group I: patients born between 23+0 and 27+6 weeks of gestation; group II: patients born within 28+0 and 31+6 weeks of gestation.

Cerebral imaging to define the grade of intraventricular haemorrhage was conducted with a cerebral ultrasound (CUS) scan at the bedside by an experienced neonatologist. They were performed on days 1, 3, 5, 7, and 10 of life and then once a week until discharge, using an Acuson 128XP (Mountainview, California) with a 7.5-MHz transducer. Standard coronal and parasagittal transfontanellar images were performed and recorded. IVH was classified according to Papile et al. [68] and the most severe degree was taken into analysis. PVL was defined according to De Vries et al. and all infants with additional brain injury (PVL and/or cerebellar lesions) were excluded from analysis.[92]

Neurodevelopmental outcome was assessed at 1,2 and 3 years of age by assessment of the Bayley Scales of Infant Development II and at the age of 5 years by Kaufmann´s

Assessment Battery for Children (K-ABC; [Melchers P (1991) and Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI) and a standardized clinical neurological examination done by an experienced staff (developmental psychologist and pediatrician). The Bayley Scales were classified as normal when psychomotor (PDI) and mental developmental index (MDI) scores were > 85 (within 2 standard deviations of reference values), K-ABC and VMI were also considered normal when > 85 (within 2 standard deviation variance).

Cerebral Palsy was defined as a nonprogressive central nervous system disorder characterized by abnormal muscle tone in at least one extremity and abnormal control of movement or posture and was defined due to location as hemiplegia, diplegia and tetraplegia. [93] Other included outcome variables were neurosensory (visual and acoustic) impairment, where need of glasses/hearing aid was defined as mild impairment and blindness/deafness were defined as severe impairment.

5.6. Neurodevelopmental Outcome Measurements

The aim of modern neonatology is not only to decrease mortality and morbidity of premature infants, but also to increase expected outcomes and quality of life for this fragile, but growing population. Developmental disabilities present a serious problem not only to the individual and their family, but also for health care systems and society. It is essential to present decision makers and involved families with relevant neurodevelopmental outcome data. Different standardised test are aviable to assess motoric, cognitive, language or psychosocial abilities. Most test acknowledge the limitations of testing before the 2nd year of life, especially in a disabled population, that is one of the reasons that recent studies extend their follow up period up to preschool age. In our IVH Study we also followed our premature infants until 5 years of age. Here are some of the test we used to in our follow up program in the neonatal follow-up clinic of the I. Department of Obstetric and Gynacology, Semmelweis University and at the Childrens and Adolescents Department at the University of Vienna.

5.6.1. Bayley Scales of Infant Development

The Bayley Scales of Infant and Toddler Development is an individually administered instrument designed to measure the developmental functioning of infants and toddlers. As a standardised and quantified test, it is very well suitable for the

neurodevelopmental assessment of risk groups of neonates, such as premature infants. With this tool we can identify developmental delay and specify areas for further intervention. Specific areas of strength or weaknesses in Motor- Cognitive- and Language Development can be separately investigated It takes about 45 – 60 minutes and consists of a series of developmental play tasks. Raw scores of successfully completed items are converted to scale scores and to composite scores. These scores are used to determine the child's performance compared with norms taken from typically developing children of their age.

The Bayley Scales of infant development were classified as normal when psychomotor (PDI) and mental developmental index (MDI) scores were >85 (± 1 SD of reference values). (Bayley N (1993) Bayley Scales of Infant Development II. Psychological Corp, San Antonio, TX)

5.6.2. Kaufmann's Assessment Battery for Children and Beery- Buktenica Developmental Test of Visual-Motor Integration

The Kaufman Assessment Battery for Children (KABC-II) is an individually administered measure of the processing and cognitive abilities of children and adolescents aged 3–18. This psychological test gives special attention to certain emerging testing needs, such as use with handicapped groups, application to problems of learning disabilities. This test specifically answers questions of strength and weaknesses and offers solutions for future intervention. It has a non-lingual component which allows professionals to test children with disabilities, which is a very important feature when caring for premature infants. Kaufman Assessment Battery for Children, Second Edition (KABC-II) The Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI; [Beery KE, Buktenica N[93]A, Beery NA (2004)Manual 5th edition for Assessment of visual-motor integration; MHS, Ontario, Canada]) is a test used at preschool age to asses neuromotor function, especially fine-motoric. The KABC and VMI are considered normal when scores are above 85 (within two standard deviation variance).

5.6.3. Gross Motor Function Classification System (GMFCS)

The Gross Motor Function Classification System (GMFCS) for Cerebral Palsy is a clinical tool designed to evaluate change in gross motor function in children with cerebral palsy. The test is based on self-initiated movement with emphasis on basic function such as

sitting, transfer and mobility. It is a very useful test as it is classified into five categories, based on functional limitations in everyday life. Children below 2 years of age should be tested at corrected age. Emphasis is laid on what the child can achieve, rather than limitations and weaknesses. [94]

LEVEL I:Infants move in and out of sitting and floor sit with both hands free to manipulate objects. Infants crawl on hands and knees, pull to stand and take steps holding on to furniture. Infants walk between 18 months and 2 years of age without the need for any assistive mobility device.

LEVEL II:Infants maintain floor sitting but may need to use their hands for support to maintain balance. Infants creep on their stomach or crawl on hands and knees. Infants may pull to stand and take steps holding on to furniture.

LEVEL III:Infants maintain floor sitting when the low back is supported. Infants roll and creep forward on their stomachs.

LEVEL IV:Infants have head control but trunk support is required for floor sitting. Infants can roll to supine and may roll to prone.

LEVEL V: Physical impairments limit voluntary control of movement. Infants are unable to maintain antigravity head and trunk postures in prone and sitting. Infants require adult assistance to roll.

5.6.4. Scheffzek Neurological Classification

This classification system, which was published by a german neurologist is based on a basic neurological examination and categorizes patients into five groups. The first group 0, has no noticeable deviation from normal; 1, minor deviation but no need for treatment; 2, deviation requiring treatment, which means an abnormality with need for special training such as mild cerebral palsy, psychological behaviour disorder but no mental retardation; or well treated seizures 3, handicap such as severe epilepsy, mental retardation or severe CP; 4, severe multi-handicap [90]

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6. Results

6.1. Hydrocephalus Study

Comparing clinical data of the two hydrocpehalus groups we found that, the mean birthweight was significantly lower in the PHVD group with 1351g as infants were younger with a mean of 28 weeks of gestation compared with the congenital hydrocephalus group, which had a mean weight of 2542g and 36th weeks of gestation.

Mean day of performance of fVEP and aEEG measurements before intervention was 2.5 (±4.3) days and 8.5 (±5.7) days after the intervention in the PHVD group. Ventricular index width exceeded the 97th percentile+4 mm in 10/17 patients (58.8%). For the remaining patients in 2/17 (11.7%) 'clinical deterioration' (increased rate of apnoeas/bradycardias, vomiting or reduction of vigilance) and in 5/17 (29.4%) rapid growth on CUS measurements and availability of neurosurgeon (eg, no neurosurgeon over the weekend) was indication for intervention although VI>97p +4 mm was not yet reached. In the congenitel hydrocephalus group the mean day of performance of neurophysiological measurements before intervention was 3,3 days and 13 days after the intervention, which was very similar to the PHVD group. Ventricular index width exceeded the 97th percentile+4 mm in all 3 patients (100%), compared with 72% in patients with PHVD. The mean day of EVD insertion was 13,3 days in congenital hydrocephalus and 24,4 days in the other group. Table 1.

The results of the last fVEPs, aEEG and CUS findings prior to and the first findings after neurosurgical intervention are presented. Only N2 latencies could be defined in all patients, P1 was visible in 10/20 patients and P2 was visible in 19/20 patients. P1 and P2 are known to appear only later during maturation; for example, median gestational age of infants with visible P1 in our cohort was 35 weeks of gestation whereas it was 30 weeks in infants with no visible P1 wave curve.

In the PHVD group statistically significant differences prior to and after neurosurgical intervention were found for N2 latencies (p=<0.001), P2 latencies (p=<0.001), P1 latencies (p=0.02), ventricular width in mm (p=>0.001), AHW (p=0.005), TOD (p=0.009), aEEG score (p=0.01) and occurrence of SWC (p=0.02). Table 2. and 3.

A statistically significant correlation was found for N2 latency prior to intervention with ventricular width prior to intervention (p= 0.01, r= -0.57), resistance index prior to intervention (p=0.03, r= -0.51), and aEEG prior to intervention (p=0.02, r= 0.54). P2 latency before intervention only showed a correlation with aEEG before intervention (p=0.02, r=0.56) and with AHW before intervention (p=0.04, r= -0.63)

N2 latency after intervention showed statistically significant correlations with aEEG after intervention (p=0.04, r= 0,49), but not with ventricular width or resistance index after intervention. In the congenital hydrocephalus group, ventricle index was greater than in the other group, and fVEP latencies especially N2 and P1 showed a significant delay.

In the congenital hydrocephalus group no significancies were calculated due to the low number of patients, but there is a strong reduction of ventricular size in all dimensions after pressure reducing intervention, as well as the reduction of fVEP latencies and aEEG scores. Complett normalization was less often compared to the PHVD group. Sleep-wake-cycling was not affected at all in this group, as it was normal all the time. The following tables contain detailed clinical and statistical information.

Table 6.1.1. Clinical characteristics of the combined study cohort. The PHVD and the patients with congenital hydrocephalus are compared.

	PHVD n=17	Congenital Hydrocephalus n=3
Birthweight g (mean ± SD)	1351 ± 940	2542±848
Gestational age wks (mean ± SD)	28.3 ± 4.4	36±3
Age in wks at EVD insertion (mean ± SD)	32.5 ± 5.1	38±3
IVH II n (%)	3 (17.6)	
IVH III n (%)	10 (58.8)	
IVH IV n (%)	4 (23.5)	
Congenital malformations		3
Days of life at EVD insertion (mean ± SD)	24.4 ± 14.3	13±12
definite VP-Shunt insertion n (%)	13 (72.2)	3 (100)

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Table 6.1.2. Results of fVEPs, aEEG and CUS prior to and after neurosurgical intenvention in PHVD patients

	Before intervention	After intervention	p-value
VEP data			
P1 latency in ms (mean ± SD)	222 ± 18.4	188 ± 18.7	**0.02**
Z-score P1 latency (mean ± SD)	1.8 ± 1.4	-0.17 ± 1.7	**0.02**
P1 latency within normal range (%)	16.6%	83.4%	
N2 latency in ms (mean ± SD)	345 ± 36.8	295 ± 39.6	**<0.001**
Z-score N2 latency (mean ± SD)	3.7 ± 1.4	1.0 ± 1.7	**<0.001**
N2 latency within normal range (%)	5.8%	58.8%	
P2 latency in ms (mean ± SD)	512 ± 59.9	421 ± 48.9	**<0.001**
Z-score P2 latency (mean ± SD)	3.8 ± 1.2	0.6 ± 1.6	**<0.001**
P2 latency within normal range (%)	0%	68.7%	
Mean Day of neurosurgical intervention (mean ± SD)	24.4 ± 14.3		
aEEG data			
aEEG score	1 ± 0.6	0.5 ± 0.7	**0.01**
aEEG normal (%)	23.5%	58.8%	
SWC present	58.8%	82.3%	**0.02**
Seizures present	29.4%	11.7%	**n.s.**
CUS data			
Ventricular width in mm (mean ± SD)	15.8 ± 3.5	11.2 ± 2.4	**<0.001**
Ventricular index > 97th percentile (%)	94.1%	47.1%	**0.005**
Ventricular index > 97th percentile + 4mm (%)	58.8%	0%	**0.002**
AHW (anterior horn width) mm (mean ± SD)	6.6 ± 2.2	3.8 ± 1.5	**0.005**
TOD (thalamo-occipital distance) mm (mean ± SD)	24.2 ± 4.5	16.5 ± 3.9	**0.009**
Resistance index (mean ± SD)	0.8 ± 0.07	0.77 ± 0.06	**n.s.**
Sedative/analgetic/anticonvulsive medication	52.9%	47%	**n.s.**

Table 6.1.3. The results of the fVEP, aEEG and CUS measurements in congenital hydrocephalus

	Before intervention	After intervention
fVEP		
P1 latency ms (mean ± SD)	265 ± 15,5	217 ± 9,1
P1 latency within normal values(%)	0%	66%
N2 latency ms (mean ± SD)	334 ± 19,1	306 ± 18,3
N2 latenciy within normal values (%)	0%	33%
P2 latency ms (mean ± SD)	459 ± 43,1	372 ± 10,6
P2 latency within normal valaues (%)	33%	66%
Mean day of intervention (átlag ± SD)	13,4 ± 13	
aEEG data		
aEEG score	0,66	0,33
aEEG normal (%)	33%	66%
SWC present	100%	100%
Seizures present	0%	0%
CUS		
ventricular width in mm (mean ± SD)	20,05 ± 6,2	15.1 ± 5,7
Ventricular index > 97th percentile (%)	0%	33%
Ventricular index > 97th percentile + 4mm (%)	100%	0%
AHW (anterior horn width) mm (mean ± SD)	9,5 ± 2,8	5,7 ±1,7
TOD (thalamo-occipital distance) mm (mean ± SD)	28,2 ± 5,2	21 ± 4,3
Resistance index (mean ± SD)	0.73 ± 0.08	0.76 ± 0.11
Sedative/analgetic/anticonvulsive medication	0%	33%

6.2. MRI-compatible Incubator Study

A total of 129 infants underwent magnetic resonance neuro-imaging during our three year study period. A significant decrease of the mean gestational age and the mean weight was observed between the two following periods. (Table 6.2.1.) The mean imaging time decreased with 4 minutes - 34,43 min without and 30,29 min with the INC - during the two periods. The whole MRI procedure was decreased with a minimum of 20 minutes, as no replacement and stabilization of the infant was necessary in the MRI. During the MRI imaging a mean of 1 additional sequence was performed in the INC group. There was a significant increase in the number of infants under 2000g examined, while the number of critically ill infants and ventilated infants increased with 18-16% respectively using the MRI-Compatible Incubator. Clinical data is listed in the table 6.2.1. below.

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Table 6.2.1.Clinical characteristics of the two study populations. Mean values and significance values are displayed.

	INC (n=99)	No INC (n=30)	p value
Mean Age (GA)	38,82	43,03	p=0.015
Mean Weight (g)	2766	3308	p=0.017
Under 2000g	28%	10%	p=0.04
Mean Imaging time (min)	30,29	34,43	p=0.113
Mean Sequences	10,63	9,67	p=0.231
Ventilation necessary during examination	36%	20%	p=0.077
Critically ill infants	48%	30%	p=0.074
Incomplete Imaging Procedure	0%	10%	p= 0.001

All infants received sedatives according to our clinical protocol before imaging. (The infants were fasting 6 hours before the examination, either chlorprothixen 1,5mg/kg p.o. 2-3h or chloralhydrate 50-80mg/kg was administered 30min before the examination, if necessary midazolam 0,1mg/kg was given at the start of imaging additionally). Due to infant instability and insufficient sedation 10% of MRI examinations were terminated incompletely or interrupted and started again without the INC, while all planned protocols were finished with the INC.

The usefulness of the MRI examination was determined by comparing the change in clinical management and the change in the initial diagnosis in each patient. More than 50% of all cases was management change initiated after MRI, or the clinical diagnosis changed or further specified. There was no significant difference between the two groups. Detailed results are presented in the table below.

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Tables.6.2.2. part 1 and 2 Change in management and diagnosis in pathological groups. The table contains the number of patients with seizures, hydrocephalus, malformations, asphyxia, PVL, IVH, infections, thrombosis, metabolic diseases, tumors and infarctions and how many of these patients ended up with different diagnosis or a change in therapy after the MR examination.

Part 1.

Primary Indication for MRI	No	Change in Diagnosis	No.	Change in Clinical Management	No.
Seizures		Malformation	4	OP / anticonvulsive	3/1
		Thrombosis	4	Anticoagulation	4
		Infarction of the middle cerebral Artery	1	Anticonvulsive therapy	1
		Parasagittal Infarction	1	No therapeutic expansion	1
		Subdural Haematoma	1		
		Hypoxic-ischaemic encephalopathy	1	Anticonvulsive therapy	1
		Abscess	1	Anticonvulsive therapy	4
		Kernicterus	1		
		Tumour	1		
	35		**15**		**15**
Hydrocephalus		Oedema+Necrosis	2	OP decision	20
		Stenosis of the Aqueduct	3		
		Lipoma compressing the Aqueduct	2		
		Parenchymal pathology	1		
		Malformation	1		
	21		**9**		**20**
Malformation		Stenosis, Hygroma of the Aqueduct	2	OP decision	8
		Lymphangioma	1	No OP	1
		Herniation	1		
		Subarachnoid bleeding	2	Anticonvulsive therapy	2
		Calcification	1		
		Extra malformation	2		
		Normal Anatomy	1		
	15		**10**		**11**
Asphyxia		No Pathology	6	Oedema therapy	3
		Thalamus bleeding	1		
		White matter abnormality	1		
		IVH	1	Prognosis	2
	13		**9**		**5**

Primary Indication for MRI	No	Change in Diagnosis	No.	Change in Clinical Management	No.
PVL		Normal	3		
		Atypical cyst formation	1		
		Polycystic malformation	1		
	10		**5**		**0**
IVH		Infarction of the Middle cerebral artery + Thrombosis	1	Anticoagulation	1
		Temporal Bleeding+AVM	1	No Neurosurgery	1
		Fracture + Parenchymal Lesion	1	Neurosurgery	1
		Asphyxiia + Subdural bleeding	1		
		Subarachnoideal bleeding	1		
	9		**5**		**3**
Infection		Necrosis	2	OP	1
		No abscess	1	Antibiotic therapy	2
		More abscess	2	Anticonvulsive therapy	1
		Extra Malformation	1	Anticonvulsive therapy	1
	7		**6**		**6**
Thrombosis		Normal	2	Anticoagulation termination	2
		Extra thrombosis in the venal sinus	1	Anticoagulation further needed	2
		Staging – old lesion	2	Anticoagulation termination	2
	6		**5**		**6**
Metabolic disease		Normal anatomy	1		
		Malformation	2	OP	1
		Metabolic Infarction (FFTS, organic aminoacidopathy)	2	Disease-specific information +therapy	2
	5		**5**		**3**
Tumour		Additional Thrombosis	2	Anticoagulation	2
		Bleeding+Thrombosis	1	OP	3
		Herniation	1		
	7		**3**		**5**
Infarction		Old infarction	1	No anticoagulation	1
	1		**1**		**1**
Sum	**139**		**73**		**75**

Most additional information was gained when infants with HIE, malformations or tumors underwent MRI. In the group of patients with seizures, but no ultrasound abnormalities MRI provided usefull information in 43%.

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Management changes were initiated included change in anticonvulsive or antibiotic therapy, anticoagulation, operative decision or withdrawal of therapy in case of severe HIE.

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6.3. Asphyxia Study

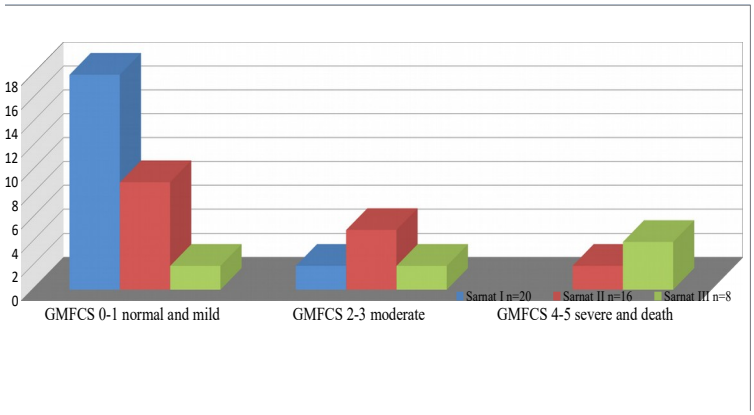
In our asphyxia study cohort the mean birthweight was 2465g , which consisted of 44 HIE patients, 24 term infants and 20 premature infants. Mean Apgar score at 5 minutes of age was 5,7, and the mean pH was 6,93. The clinical characteristics of the study population are listed in table 6.4.1. The mean gestational age of the term group was 39 weeks of gestation and it was 32 weeks in the preterm group.

Table 6.3.1 Clinical and epidemiological data. Mean values and standard deviations of postmenstrual age, birthweight Apgar and pH are displayed.

	Mean	Standard Deviation
PMA	35,52	±3,94
Birthweight	2465,59	±801,34
Apgar5	5,71	±2,12
pH	6,93	±0,21
Sarnat I. (%) Sarnat II. Sarnat III.	45,4 36,4 18,2	

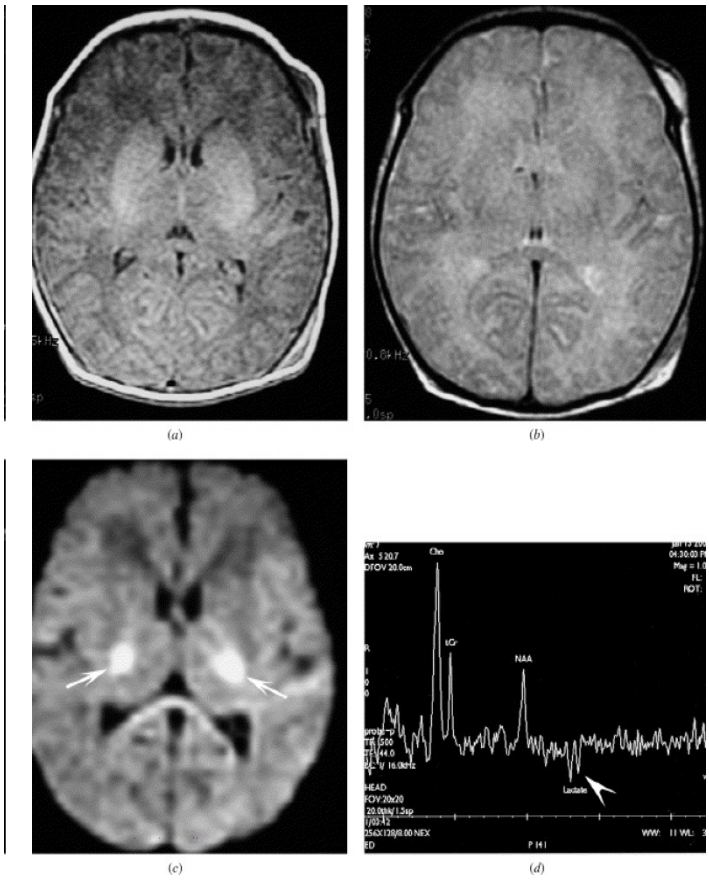
Most of our infants 45,4% had a mild neonatal encephalopathy, 36,4% moderate and 18,2% had severe encephalopathy according to the clinical criteria by Sarnat and Sarnat 1976 and the aEEG pattern according to Hellström-Westas. The neurodeveopmental outcome at two years of age was in 68% normal or mildly abnormal, 14% had severe disability or died and 18% had a moderate outcome.

Graph 6.3.1: Correlation of Sarnat scores and neurodevelopemental outcomes.



The following figure shows the MRI results of an asphyxiated infant with severe neonatal encephalopathy.

Figure 6.3.1. MRI Images of a patient with HIE. a: T1 weight image with increased signal intensities in thalamus, putamen and PLIC, b: brain oedema c: diffusion tensor imaging with increased diffusion in thalamus, d: proton spectroscopy with prominent lactate peak.



We have found a significant positive correlation between neurodevelopmental outcome at two years of age for both MRI and aEEG scores. The aEEG had a better prognostic value when the measurement was within two days after birth, while in contrast late MRI (after the first week) showed stronger correlations with good neurodevelopmental outcome. The positive predictive value of the MRI score was 88% while the negative predicitive value was 83%. The aEEG had a positive predictive value of 92% und negative predictive value war 89% in our study cohort.

There was no significant correlation of CUS images or Doppler Resistance Index measurements with neurodevelopmental outcome. Clinical data showed no significant

correlation either. The presence of seizure activity at all times had a strong correlation but no significance with unfavourable neurodevelopmental outcome. ($\mathbf{r^2}$=0,7)

We developed a combined variable from the MRI Score, which showed the involvement of four regions (Thalamus, PlIC, White Matter, Cortical Gray matter) and diffusion abnormalities (0-5) with the aEEG scores that ranged from normal to severely abnormal (0-4), where higher scores were for more severe cases. This combined variable that we called MRI+aEEG Score showed a very strong positive correlation with favourable outcome and was highly significant.(r^2=0,81) This was superior to all, but one single parameter, as the late MRI had the highest correlation with neurodevelopmental outcome.

Table 6.3.3. Correlations of clinical, neurophysiological and imaging data with neurodevelopmental outcome

	Good Outcome (r^2)	P=
APGAR 5min	-0,398	0,082
pH	-0,393	0,107
aEEG 1-2.day of life	0,789	< 0,0001
aEEG second week	0,638	< 0,0001
MRI Score	0,698	0,001
MRI within 7 days	0,531	0,062
MRI after 7 days	0,925	< 0,0001
Seizures	0,383	0,096
CUS and RI	0,251	0,315
MRI+aEEG Score	0,806	0,000

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6.4. Mismatch Negativity Study

Our goal was to test the use of phoneme and stress information in infancy at the word level. In our experiment the suprasegmental cues are as complex as in spoken utterances, so that the stimuli used are highly similar to the typical stress pattern of the Hungarian language (changing intensity, F0, and rise time). We presented a meaningful word ’Banán’ and derived a meaningless phoneme deviant ’Panán’ (zöngés-zöngétlen pár) in the phoneme condition. Int he stress condition, word stress was on the second syllable (ba-nán). Our study population included 21 preterm and 25 full term neonates, examined at 6 or 10 months of age.

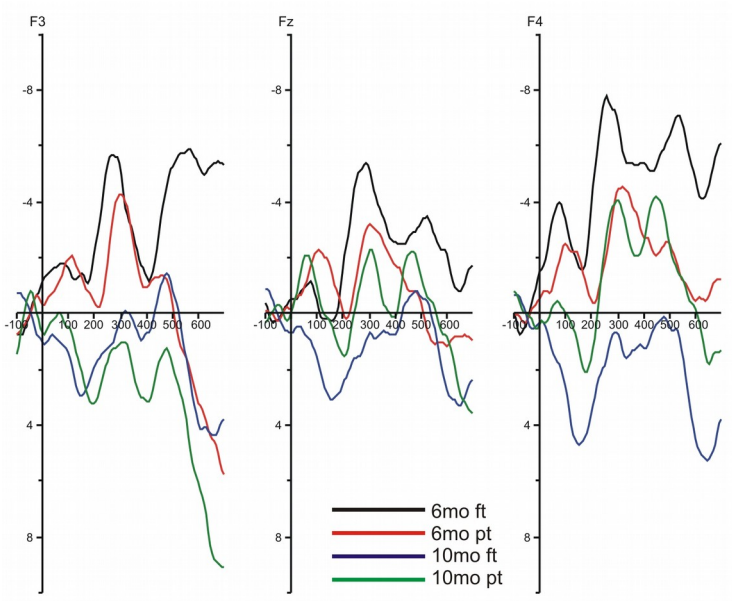
Table 6.4.1. Clinical characteristics of the four groups of subjects. PT: preterm; FT: full-term; SE: standard error at 6 and 10 month of age.

	N	Mean age in month (SE)	Birth weight in gram (SE)	GA in week (SE)
6PT	10	6.2 (.29)	1632 (212.27)	31.8 (1.009)
6FT	10	6.05 (.28)	3373 (113.99)	39.6 (0.371)
10PT	11	10.09 (.21)	1288.18 (112.89)	30.64 (.54)
10FT	15	10.73 (.61)	3312.67 (111.46)	39.2 (.341)

1. In case of phoneme deviant we can see the two areas where the deviant curve differs from the standard one. This twofold difference is partly similar to the previous results in adults, where our study group found two negativities. The first was located at 300 ms and a second one at 400 ms. [91]

The following graph shows the comparison of the different age groups using the grand average curves. In the first time window between 250-300ms, results show that 6 month-olds had bigger MMN than the 10 month-olds but the preterm group didn't differ from the full-term group. In the second time window (500 ms) the MMN this time was also bigger in case of 6 month-olds than 10 month-olds, but the difference was not significant.

Graph.6.4.2. Grand Averages of MMN of the Phoneme condition.



Summarizing the results obtained in the phoneme deviant condition in respect to the question of maturation there are only age related differences in case of the first time window. Two MMN components were present to the phoneme deviant in all the four groups. We could find differences only in case of the first component by age: younger infants had bigger MMN to the phoneme deviant than the older infants, although this difference was not significant. No differences were found between the preterm and full-term groups.

2. In case of the stress deviant condition all the infants detected the presence of stress (S+) in the middle of the acoustic stimuli, synchronized to the extra stress cue on the second syllable. When the two time windows were analised separetly, in the first time window (300-350ms) we did not find a significant main effect of condition. In the second time window (500-550ms) a significant main effect of status was found. Preterm infants had smaller positive mismatch responses than those of the full-term group. Younger infants had bigger positive MMR than the older group.

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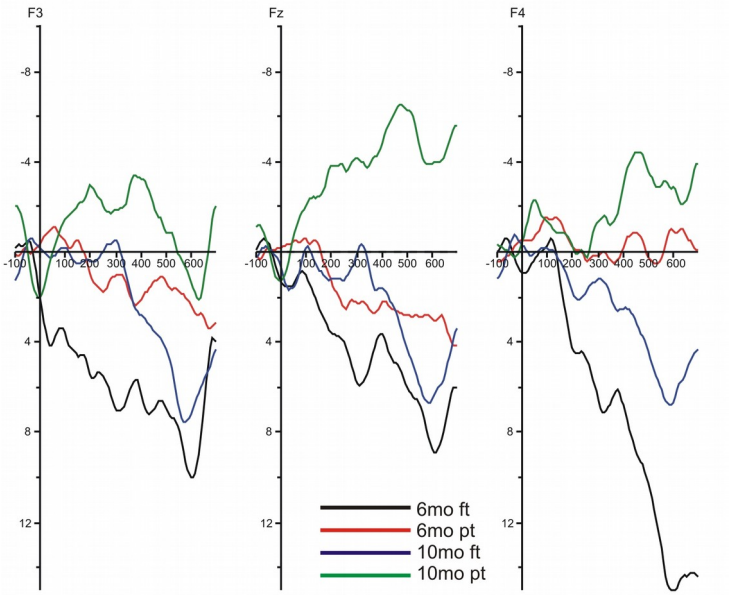
The following graph shows the grand average results for the different patient groups for the stress deviant condition.

Graph 6.4.3. Grand averages of MMN of the Stress Condition



Summarizing the results we can argue that in case of natural speech stimuli and complex stress cues, the detection of suprasegmental speech cues is based on detecting the presence of the salient acoustic change. Infants didn't detect the absence of the stress in the first time window as adults did in our previous experiment. We found a positive MMR in the second time window. Here we did found differences between the preterm and full-term groups as the former had significantly smaller mismatch responses than the latter. As the difference between age groups was not significant we can conclude that infants at the age of 6 months are able to use stress information, but there is a significant difference in processing between preterm and fullterm infants.

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6.5. IVH Study

The study group included 471 preterm infants born below 32 weeks` gestation; 184 developed an IVH; in 33 infants additional cerebral injuries (PVL, cerebellar lesions) were found and therefore they were excluded from the analyis. 37 of the remaining 151 infants 37/151 (24,6%) developed an IVH grade I, 84/151 (55,6%) an IVH grade II, 18/151 (11,9%) an IVH grade III and 12/151 (7,9%) an IVH grade IV. Group I. had 121 patients with low grade IVH and Group II. had 30 patients with high grade IVH. Table 6.5.1. shows that premature infants with IVH were smaller and younger, than the healthy control patients and had significantly more morbidities, such as CLD, RDS, PDA, ROP and of course PHH. Delivery was more often vaginal in the IVH group suggesting severe threatening premature birth, where there was less time for an elective cesarian section, although the rate of cesarian section was very high in the whole group of patients under 32 weeks of gestation with 85,3%.

Table 6.5.1.: Clinical characteristics of the total study group

		no IVH n= 320	IVH n=151	*p*-value
Gestational age (weeks):		27,8	26,7	<.0001
Birth weight (grams):		1037 +- 256	910 +- 244	<.0001
Antenatal steroids % (n)		94,7 (302)	88,7 (134)	0,01
Sex % (n):	female	45,9 (147)	48,3 (73)	ns
Multiple birth % (n)	yes	27,3 (90)	19,6 (36)	0,04
Mode of delivery % (n)	vaginal	11,6 (37)	21,2 (32)	0,02
RDS % (n)	yes	57,2 (183)	80,8 (122)	0,002
CLD % (n)	yes	8,8 (28)	31,3 (47)	0,03
PDA % (n)	yes	14,4 (46)	33,1 (50)	0,001
NEC % (n)	yes	8,8 (28)	13,2 (20)	ns
AIS % (n)	yes	31,6 (101)	51,7 (78)	<0,001
PHH % (n)	yes	0	20,5 (31)	<0,001
Shunt % (n)	yes	0	13,9 (21)	<0,001
ROP % (n)	yes	6,3 (20)	19,2 (29)	<.0001

As shown in table 6.5.2 and 6.5.3. there is a significant increase of abnormal results with increasing grade of IVH. Whereas the high percentage of abnormal results remained nearly unchanged in patients with IVH grade III and IV at higher ages, abnormal results of

patients with IVH grade I and II significantly increased over time in group II, but decreased or stayed the same in group I. It is also very important to notice that this premature group has impaired cognitive and motor outcomes, such as 10%CP and 17% abnormal Bayley Scales at three years of age even with no IVH.

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Table 6.5.2 Outcome parameter of patients of Group I with regard to IVH grade

Outcome Parameter	No IVH	IVH I	p-value	IVH II	p-value	IVH III	p-value	IVH IV	p-value
Outcome at 1 year									
PDI < 70	16,2%	43,4%		40%		77,8%		90%	
MDI < 70	10,6%	8,6%		27,7%		78,8%		66,7%	
PDI mean ± SD	84,6 +- 17,0	76,3 +- 20,8	0,03	69,4 +- 18,8	<0,01	59,3 +- 19,9	<0,01	53,6 +- 11,4	<0,01
MDI mean +- SD	88,9 +- 15,1	85,7 +- 10,6	n.s.	78,1 +- 18,5	<0,01	63,0 +- 23,3	<0,01	59,0 +- 15,7	<0,01
Outcome at 2 years									
PDI < 70	13,4%	15%		30,9%		75%		66,7%	
MDI < 70	11,4%	35%		28%		75%		50%	
PDI mean +- SD	89,8 +- 16,5	84,4 +- 14,8	n.s.	82,1 +- 22,0	0,01	61,8 +- 21,9	<0,01	62,1 +- 16,2	<0,01
MDI mean +- SD	90,0 +- 17,8	81,1 +- 18,0	0,03	82,6 +- 19,3	0,02	62,1 +- 23,6	<0,01	68,1 +- 21,3	<0,01
Outcome at 3,5 years									
PDI < 70	17,3%	42,8%		23,2%		70%		85,7%	
MDI < 70	17,5%	23,8%		20,4%		60%		62,5%	
PDI mean +- SD	84,8 +- 17,3	74,8 +- 19,6	0,01	82,9 +- 20,4	n.s	57,7 +- 30,0	<0,01	53,4 +- 11,7	<0,01
MDI mean +- SD	86,1 +- 19,1	80,1 +- 17,7	n.s.	84,2 +- 19,1	n.s	61,8 +- 30,7	<0,01	64,5 +- 23,3	<0,01
Outcome at 5,5 years									
KABC < 70	7,6%	6,3%		12,9%		33,3%		50%	
KABC mean ± SD	91,5 ± 15,1	90,4 ± 13,6	n.s.	86,2 ± 16,2	n.s.	88,6 ± 11,1	n.s.	88,5 ± 10,6	n.s
VMI mean ± SD	92,7 ± 20,0	93,5 ± 14,6	n.s.	93 ± 15,6	n.s.	67,5 ± 14	0,04	76 ± 26,8	0,04
Cerebral palsy	14,3%	34,8%	0,01	55%	<0,01	63,6%	<0,01	90,9%	<0,01
Hemiplegia	1/25	0%		1/33		0%		0%	
Diplegia	22/25	7/8		27/33		7/9		6/14	
Tetraplegia	2/25	1/8		5/33		2/9		8/14	
Visual impairment	7,5%	26,1%	<0,01	27%	<0,01	45,5%	0,03	90,9%	<0,01
Acoustic impairment	2,2%	0%	n.s	3,2%	n.s	0%	n.s	0%	n.s

Table 6.5.2: Group I: patients born 23+0-27+6 weeks' gestational age; IVH = intraventricular hemorrhage; PDI= psychomotor developmental index (Bayley Scales of Infant Development); MDI = mental developmental index (Bayley Scales of Infant Development); KABC= Kaufmann´s Assessment Battery for Children; N < 70 = number of patients with developmental index below 70, VMI = Visual Motor Integration; Visual and acoustic impairment = including mild and severe forms of impairment

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Table 6.5.3 Outcome parameter of patients of Group II with regard to IVH grade

Outcome Parameter	No IVH	IVH I	p-value	IVH II	p-value	IVH III	p-value	IVH IV	p-value
Outcome at 1 year									
PDI < 70	13,6%	33,4%		30%		0%		100%	
MDI < 70	8,3%	0%		20%		25%		75%	
PDI mean ± SD	87,2 +- 16,8	74,6 +- 16,6	n.s.	75,1 +- 14,7	0,03	79,0 +- 6,9	0,04	49,3 +- 0,5	<0,01
MDI mean +- SD	91,2 +- 13,4	92,1 +- 11,6	n.s.	84,7 +- 16,6	n.s.	79,5 +- 7,5	0,04	49,3 +- 0,5	<0,01
Outcome at 2 years									
PDI < 70	11,7%	20%		10%		25%		100%	
MDI < 70	11,7%	0%		10%		0%		75%	
PDI mean +- SD	91,2 +- 17,9	86,8 +- 21,4	n.s.	90,9 +- 12,2	n.s.	81,5 +- 13,2	n.s.	49 +-0	<0,01
MDI mean +- SD	92+-19	89+-21	n.s.	98+-17	n.s	98+- 15	n.s.	56+-10,6	<0,01
Outcome at 3,5 years									
PDI < 70	7,1%	20%		14,3%		50%		100%	
MDI < 70	9%	0%		7,1%		25%		75%	
PDI mean +- SD	92,0 +- 17,1	92,2 +- 7,2	n.s.	84,1 +- 11,9	0,03	69,0 +- 22,4	0,01	49 +- 0	<0,01
MDI mean +- SD	93,9 +- 19,2	104 +- 7,5	n.s.	95,5 +- 10,9	n.s	89,7 +- 18,5	n.s.	56,5 +- 10,6	<0,01
Outcome at 5,5 years									
KABC < 70	1,4%	0%		0%		0%		50%	
KABC mean ± SD	98,7 ± 13,0	112,5 ± 2,5	n.s.	97,8 ± 9,1	n.s.	81,6 ± 5,8	0,02	55 ± 8	<0,01
VMI mean ± SD	107 ± 13,6	105 ± 14,6	n.s.	83 ± 15,6	n.s.	88 ± 14	0,02	not available	
Cerebral palsy	8,5%	12,5%	0,01	23,5%	0,01	60%	<0,01	100%	<0,01
Hemiplegia	1/9	0%		2/4		0%		0	
Diplegia	6/9	7/8		2/4		2/3		1/4	
Tetraplegia	2/9	1/8		0		1/3		3/4	
Visual impairment	3,3%	0%	n.s.	0%	n.s.	20%	0,04	100%	<0,01
Acoustic impairment	1,7%	0%	n.s	0%	n.s	0%	n.s	25%	n.s

Table 6.5.3: Group II: patients born 28+0-31+6 weeks' gestational age; IVH = intraventricular hemorrhage; PDI= psychomotor developmental index (Bayley Scales of Infant Development); MDI = mental developmental index (Bayley Scales of Infant Development); KABC= Kaufmann´s Assessment Battery for Children; N < 70 = number of

patients with developmental index below 70, VMI = Visual Motor Integration; Visual and acoustic impairment = including mild and severe forms of impairment

As the main interest of this study was the effect of low grade haemorrhage (IVH I and II) on neurodevelopmental outcome, especially in the extreme premature infant, we analised our patients separatelywith low grade IVH in group I. under 28 weeks of gestation and group II. born above 28 weeks of gestation. results are shown here in the following table 6.5.4..

Table 6.5.4. Outcome parameter of all patients with IVH I and II in comparison with the two different age groups

Outcome Parameter	No IVH Group I	No IVH Group II	p-value	IVH I Group I	IVH I Group II	p-value	IVH II Group I	IVH II Group II	p-value
Outcome 1 year									
PDI < 70	16,2%	13,6%		43,4%	33,4%		40%	30%	
MDI < 70	10,3%	8,3%		8,6%	0%		27,7%	20%	
PDI	84 +- 17	74 +- 16	n.s.	76 +- 20	74 +- 16	n.s.	69 +- 18	75 +-14	n.s
MDI	88 +- 15	92 +-11	n.s.	85 +- 10	92 +- 11	n.s.	78 +- 18	84 +-16	n.s.
Outcome 2 years									
PDI < 70	13,4%	11,7%		15%	20%		30,9%	10%	
MDI < 70	11,4%	11,7%		35%	0%		28%	10%	
PDI	89 +- 16	91 +- 17	n.s.	84 +- 14	86 +- 21	n.s.	82 +- 22	90 +- 12	n.s.
MDI	90 +- 17	92 +- 19	n.s.	81 +- 18	89 +- 21	n.s.	82 +- 19	98 + 17	0,02
Outcome 3,5 years									
PDI < 70	17,3%	7,1%		42,8%	20%		23,2%	14,3%	
MDI < 70	17,5%	9%%		23,8%	0%		20,4%	7,1%	
PDI	84 +- 17	92 +- 17,1	<0,01	74 +- 19	92 +- 7	<0,01	82+- 20	84+- 11	n.s.
MDI	86 +- 19	93 +- 19	<0,01	80 +-17	104 +- 7	<0,01	84 +- 19	95 +-10	<0,01
Outcome 5,5 years									
KABC < 70	7,6%	1,4%		6,3%	0%		12,9%	0%	
KABC	91 ± 15	98 ± 13	<0,01	90 ± 13	112 ± 2	<0,01	86 ± 16	97 ± 9	n.s
VMI	92 ± 20	107 ± 13	<0,01	93 ± 14	105 ±14		93 ± 15	83 ± 15	n.s.
CP	14,3%	8,5%	n.s.	34,8%	12,5%	<0,01	55%	23,5%	0,01
Hemiplegia	1/25	1/9		0/8	2/4		1/33	2/4	
Diplegia	22/25	6/9		7/8	2/4		27/33	2/4	
Tetraplegia	2/25	2/8		1/8	0		5/33	0	
Visual impairment	7,5%	3,3%	n.s.	26,1%	0%	0,01	27%	0%	<0,01
Acoustic impairment	1,5%	1,7%	n.s	0%	0%	n.s	3,2%	0%	n.s

Table 6.5.4.: Group I = patients born within 23+0-27+6 weeks of gestation; Group II: patients born 28+0-31+6 weeks' gestational age; IVH = intraventricularhemorrhage; PDI= psychomotor developmental index (Bayley Scales of Infant Development); MDI = mental developmental index (Bayley Scales of Infant Development); KABC= Kaufmann´s Assessment Battery for Children; N < 70 = number of patients with developmental index below 70, VMI = Visual Motor Integration; Visual and acoustic impairment = including mild and severe forms of impairment

Predicted probabilities for impaired outcome in dependence on GA and IVH show that the interaction between GA and IVH was significant (p 0.01). This effect is even more significant in mild IVH grades (I and II) compared to severe grades of IVH (grades III and IV).

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7. Discussion

7.1. Hydrocephalus Study

This study demonstrates the impact of ventricular dilatation and subsequent CSF drainage on fVEPs, aEEG and CUS measurements. The mean day of performance of fVEP and aEEG measurements in the congenital hydrocpehalus group before intervention was 3,3 days and 13 days after the intervention, which was similar to the IVH group with 2,5 day and 8,4 days respectively. In contrast neurosurgical intervention with EVD needed to be implanted significantly earlier in the congenital group at the 13th day of life in comparison with the 24th day of life in the PHVD group. We suggest that these differences are due to the fact, that elevated intracranial pressures are significantly longer present in the congenital group, with extremely dilated ventricles. Also intraventricular haemorrhage develops on average on the third day of life and it takes about 1-3 weeks until it causes hydrocephalus and the intraventricular dilatation becomes symptomatic.

Whereas ventricle width exceeded 97th percentile + 4mm in 58.8% of patients only, all patients (100%) showed a delay of their P2 latency and almost all patients (94.2 %) a delay of their N2 latency at fVEPs at the time of CSF drainage. After drainage, P2 latency was within normal range in 68.7% of patients and N2 latency in 58.8% of patients with the mean of 8.5 days. N2 latencies prior to intervention correlated significantly with ventricular width prior to intervention ($p= 0.01$), resistance index prior to intervention ($p=0.03$), and aEEG scores prior to intervention ($p=0.02$). N2 latencies after intervention showed statistically significant correlations with aEEG scores after intervention ($p=0.04$), but not with ventricular width or resistance index after intervention. P2 latencies before intervention only showed a correlation with changes in aEEG before intervention ($p=0.02$) and with AHW ($p=0.04$) before intervention. We can conclude from these results that elevated intracranial pressure results in impaired brain function, represented by the fVEP delay and the pathological aEEG before intervention, which showed a strong correlation with ventricular dilatation. As there was no significant correlation between ventricular size and neurophysiological measurements after the intervention, only between fVEP and aEEG measurements, we hypothetise that the sudden reduction of intracranial pressure results

quickly in normal ventricule size, but brain function needs an adaptive period in order to normalize.

In a previous publication from our research group we could demonstrate that aEEG indicates impaired cerebral function with progressive PHVD before clinical deterioration occurs and before CUS measurements indicate the need for neurosurgical intervention.[95] We could reproduce our findings in the present study showing that only 23.5% of all infants showed a normal aEEG trace prior to intervention, but 58.8% after intervention. This time we added fVEP-findings and showed that fVEP is an additional functional method available and feasible in these patients, which allows us to optimize timing of the CSF drainage procedure even further. Similar to our aEEG and fVEP results, Soul and coworkers used near-infrared-spectroscopy to show that CSF removal in infants with PHVD lead to significant increases in cerebral perfusion, cerebral blood volume and oxidative metabolism. [46, 96]

Ventriculomegaly in PHVD is thought to compress the adjacent white matter first and later on also the cortical grey matter. As previously postulated suppressed aEEG-acitivity might be a sign of reduced blood flow and/or compression of intracranial structures. Since the same aEEG changes were present in this study this strengthens our hypothesis. With regard to our fVEP findings we hypothesize that periventricular white matter structures (such as the visual pathway) show signs of impairment even earlier when compared to measurements of cortical activity using aEEG. Similar findings have been published in a previous study by Pierrat and coworkers using somatosensory and visual evoked potentials showing a delay in latency during progressive PHVD and normalisation after shunt insertion.[97]

This observation is supported by our findings as fVEP latencies were the most sensitive marker for impairment of cerebral function. All of our study patients (100%) showed abnormal fVEP latencies prior to CSF drainage procedures, whereas aEEG-activity was abnormal in only 76.5%.

Comparing PHVD with congenital hydrocephalus, we found that although neurosurgical intervention reduced the size of ventricles and fVEP latencies were less delayed, there was only one case (33%) where they normalised completely, in contrast with 58-83% (N2,P1 respectively) in PHVD patients. We hypothetise that this differenece is not

only due to the direct changes in visual neural pathways in congenital anomalies, but also due to the fact that elevated intracranial pressure have been significantly longer present. fVEPs measurement are even in this population usefull indicators of intracranial pressures and brain function.

Further potential causes explaining fVEP and aEEG changes need to be discussed. As PHVD is mainly found in severe IVH, it is difficult to delineate the influence of this underlying pathology on cerebral activity assessed using aEEG and fVEPs. Our data show full recovery (within one week) after CSF drainage in 58.8% of the patients with regard to N2-latencies and aEEG-activity, which demonstrates that impairment of cerebral function was reversible as measured with aEEG and fVEP. This observation can most likely be explained by ventricular enlargement and increased intracranial pressure rather than with the underlying irreversible pathology.

The deterioration of both methods prior to CSF drainage could also be due to an increased administration of sedative, analgetic and/or anticonvulsive medication with progressive PHVD. In our study cohort there was no difference in the use of the amount of potentially depressing medication prior to and after neurosurgical intervention.

Furthermore, aEEG and fVEPs change significantly during maturation. Therefore, it can also be postulated that the improvement/change of fVEP latencies and aEEG scores is due to maturational changes. Since major normalization of fVEP latencies and aEEG scores occurred within a mean of 8.5 days after intervention, these changes are much better explained by the consecutive pressure relief than by maturation alone. Also, with regard to VEPs maturational change is defined by latency changes of about 5ms / week, while our measurements show a decrease of 34 ms within one week of intervention. [84]

The optimal timing of intervention in PHVD remains a matter of discussion. Multiple parameters (mostly used: bulging fontanel, increasing suture width, increasing head circumference, increasing ventricular width and ventricular index) are used to define the necessity of a CSF removing intervention. All these parameters appear late in the clinical course of such patients whereas it would be most desirable to detect an impairment of cerebral function while it is still reversible.

Del Bigio and coworkers used a rat model to show a reversible collapse of capillaries in the periventricular neuropil, when shunting was performed one week after

induced hydrocephalus compared to eight weeks after ventricular dilatation. [98] The same group later showed that compensatory myelination was possible in young rats with induced hydrocephalus, if treatment was instituted prior to axonal injury. [99]

Also, in humans, a retrospective Dutch study demonstrated that early intervention (defined as time of onset of treatment when ventricular width was less then 97th percentile +4mm) was associated with a reduced risk of VP shunting. [100] Furthermore, infants receiving late treatment (once ventricular width had exceeded 97th percentile +4mm) were more likely to develop moderate to severe handicap, although recent data could not support these findings. [101]

We propose that functional methods such as aEEG and fVEPs should be used in the assessment and management of PHVD since morphological (=imaging) methods are not providing enough information. These methods offer valuable additional information about cerebral impairment and might help optimizing the timing of decompressing interventions.

7.2. MRI-compatible Incubator Study

The aim of all the neonatologists is to minimize the postnatal and treat perinatal neurological insults of the premature population, in order to achieve the best possible outcome for these infants. During a three year period when an MRI-compatible incubator was used during the second 18 months, the number of neuro-imaging examinations in newborns and premature infants more than tripled. The availability of the INC led to a significant decrease in average weight and age of the infants examined. Especially the number of infants under 2000 gram increased.

The average imaging time decreased with 4 minutes using the INC and we were able to add an additional imaging sequence as well. The whole time away from the NICU decreased with 24 minutes without the need of repositioning and stabilising the infants at the radiology department.

The built in ventillator enabled neonatologists to observe the imaging process, instead of crawling into the MRI and hand ventillating the infants, which is not only less effective, leads to suttle movements and therefore worse image quality but also more dangerous as well. The incubator is confortable and effective for patients and neonatologist and provides high quality MRI data without significant movement artefacts.

Although more critically ill infants were examined with the INC, the fitted head-coil and ear-shields contributed to a continuous examination in all cases and no additional sedative was necessary. In contrast, without the INC, 10% of MRI examinations had to be terminated prematurely, due to the instability and insufficient sedation of the infant. Recent studies suggest that MRI is possible without the use of additional sedatives. [102] Significantly shorter protocols are used in these publications, 10 minutes compared to our 30 minutes protocol, where shorter sequences might lead to limited image quality and imaging information and are only adviced in extremely unstable infants.

We analyzed the effect of the MRI examination on our everyday clinical practice for first line caregivers. Combining both periods, management changes were initiated in 58%, while in 57% of cases the initial ultrasound diagnosis was changed or further specified. These results emphasize the utility of MRI examination itself and the use of the INC with respect to patient management as younger and more instable infants have a better chance for more specific treatment.

The management was always changed in suspected diagnostic groups such as: thrombosis, metabolic disease, and conditions requiring surgery, such as PHH, trauma and certain malformations and tumours. In cases of IVH, PVL, infection, and infarcts the imaging had less effect on acute decision making as previous clinical and ultrasound diagnosis was more frequently adequate, but several studies underline their importance in later neuro-developmental prognosis.[103] [60, 71, 104-106]

In the largest group, patients with clinical seizures and unspecific cranial ultrasound, in 42% the MRI examination found a causative cranial pathology, which changed the previous treatment procedure in also 42%. Ultrasound imaging has well known limitations in detecting abnormalities of the posterior Fossa, neural migration anomalies and unspecific hyperechogenic lesions can be further identified with the MRI. Ment et al. described the importance of the identification of imaging biomarkers in the premature infant in order to better understand the background of cortical development, connectivity and early neurological injury and its correlation to neurodevelopmental outcome. They underline the importance of MRI based biomarkers such as diffusion tensor imaging, functional MRI and voxel-based morphometry in strategies for therapeutic intervention, individualised treatment and long-term neurodevelopmental risk assessment.

We can conclude that similarly to other studies, MR imaging is superior to ultrasound imaging in the premature population especially in critically ill and very low bitrhweight infants and this population benefits mostly, when an INC is available.

7.3. Asphyxia Study

HIE has always been an unexpected, devastating event for parents, neonatologist and obstetritians and presents a serious acute problem, with chronic consequences. The incidence of HIE has been stable despite joint efforts of caregivers, but the recently standardised treament modality of hypothermia has increased favourable outcome in the moderate group of asphyxiated infants.

We have demonstrated with our study of asphyxiated infants without hypothermia treatment, that neurophysiological methods such as aEEG and cerebral imaging with MRI are reliable tools in the prognosis of HIE in both term and preterm infants. 44 infants were eligable for inclusion, 24 were term neonates with HIE and 20 were premature infants from 27-36 weeks of gestation (mean 32 weeks of gestation) who also developed hypoxic ischeamic encephalopathie after birth. We found that 20 patients were in Sarnat I. stadium (46%), 16 in Sarnat II. (36%) and 8 in the most severe group with Sarnat III. (18%) Our primary outcome variable was neurodevelopmental outcome at two years of age, measured with two methods (GMFCS and Bayley scales of infant development). Comparing our results with the study of Twoney and coworkes, we found similar percent of favourable outcomes with 46% at two years of age, while in their smaller study population they had 57%. [107] An other study from Rutherford et al found higher percent of disability and deaths in preterm infants with HIE, as only 33% of their group had a favourable outcome. [108]

We have found that early aEEG (within 2 days after birth) had a better prognostic value, than later neurophysiologic measurements. There is sufficient data that suggest that aEEG has a good prognostic value in perinatal asphyxia. Severely abnormal aEEG patterns during the first two days of life had a negative prognostic value. Spontaneous changes to normal patterns such as continuous or discountinuous patterns were related to favourable outcomes. The sooner the abnormalities on aEEG disappeared, the better the prognosis was and this study found no significant correlation after 48 hours. [74] We could replicate similar results in our study, although we have found weak correlations between good

outcome and later aEEG measurements additionally. Amplitude-integrated electroencephalogram (aEEG) at <6 hours is the best single outcome predictor in term infants with perinatal asphyxia at normothermia according to Hellström-Westas. They could not replicate these results in a large study population with hypothermia. [109] The time to normal pattern showed no significant correlation with good outcome, the only moderately predictive aEEG characteristic was the time to develop Sleep-wake-cycling. Csekő et al could also demonstrate in their recent publication, that hypothermia influences the prognostic value of aEEG. They found that after 48 hours it has a significant PPV for good outcome. [110] Massaro et al found that persisting aEEG background abnormality beyond 48 h of life and lack of SWC over the course of hypothermia is predictive of adverse NICU outcome in encephalopathic newborns. [111] In our study population the presence of seizure activity showed a strong correlation with unfavourable neurodevelopmental outcome, although it was not significant.

Recent data suggests that other neurophysiological methods such as somatosensory evoked potentials (SEP) are also usefull in the prediction of neuromotor outcome. [112] This study also found in contrast with others, that only the absence of multiple seizures was associated with a normal neurocognitive outcome at school age. They found strong correlations between early childhood and school age outcome, which enables us to compare our outcome results, as we were only able to follow our patients up to two years of age.

The analysis of our MRI data suggested that MRI is a very strong outcome predictor in infants with HIE and its sensitivity increases especially after the first week of life. (0,75-0,85) Similar results have been published in a review article by van Laerhoven , where diffusion tensor imaging had a high specificity with (0.89) and T1/T2-weighed imaging had a high sensitivity of 0,98. [113]

We developed a single outcome variable combining early aEEG scores and late MRI scores. This proved to be a good method to increase the correlations with neurodevelopmental outcome, as they showed a strong significant correlation (r^2=0,8) with favourable neurodevelopmental outcome at two years of age. The combination of these two methods provide an even better tool for everyday practice of parent consultation and clinical decision making ont he neonatal ward.

A metaanalysis from Thayyil showed that for predicting adverse outcome, with conventional MRI during the neonatal period (days 1–30) had a pooled sensitivity of 91% and specificity of 51%. [114] They compared late MRI (days 8–30) with early MRI (days 1–7), and found that late MRI had higher sensitivity but lower specificity than early MRI. The main message of this study was that proton MRI spectroscopy in deep gray matter lactate/N-acetyl aspartate (Lac/NAA) peak-area ratio gave early and most specific answers for prognosis. This is unfortunately extremely important at the NICU, where clinical decision making and withdrawal of care in severe asphyxiated infants takes place on the 3-4th day of life. End of life decisions present a complex but not so rare problem on the neonatal ward. As our data also suggests MRI is a more sensible predictor of neurodevelopmental outcome after this period. We hypothetise that with the help of additional proton spectroscopy sequences the negative predicitve value can be increased during the first three days of life and should be used in severe and moderate cases of HIE in routine protocols. According to Wilkinson et al. currently MRI biomarkers alone are not sufficiently accurate to direct treatment-limitation decisions. [115] Doormal et al suggest that levels of Cho and Lact measured in the grey matter are the most indicative of survival in case of perinatal asphyxia. [116]

In our study we found similarly that late MRI was better than early in the prognosis of neurodevelopmental outcome, with high specifity especially after the 7th day of life. MRI imaging protocoll included only DTI, T1/T2-weighed imaging and only in limited cases spectroscopy. Recent data suggests that even this method is influenced by the effect of hypothermia, as diffusion abnormalities are slowed down by cooling. [117]

Our study demostrated that the timing of neurophysiological and neuroimaging methods are essential in the prognosis of asphyxiated infants. The combination of early aEEG and late MRI proved to be sensitive markers for neurodevelopmental outcome at two years of age. New methods such as Proton MRI sequences or SEP-s could further define outcome data and enable neonatologists and parents to make better and more informed treatment decisions in this difficult patient population.

7.4. Mismatch Negativity-Study

In the present study we applied a MMN ERP paradigm in an acoustic odd-ball study, where we used a meaningful word as a standard stimulus, and two deviants: a pseudo-word by changing the first consonant of the standard as a phoneme deviant, and stress deviant by moving the stress to the second syllable which violates the highly regular Hungarian stress rule. In order to test the role of intrauterine experience in prosodic processing we compared full-term and healthy preterm infants at two ages, 6 months and 10 months.

Neural representations of phonemes belonging to a child's native language begin to be laid down within the first year of life. At birth, human infants are able to detect any phonemic difference independent of language. However, between 6 months and 12 months of age the developing brain begins to respond preferentially to phonemes inherent to the infant's native language and simultaneously begins to lose the ability to discriminate between nonnative phonemes. [118, 119] Studies using event related brain potentials as a measurement of developmental changes use the passive oddball paradigm where the deviant stimuli are expected to elicit the Mismatch Negativity ERP component. This paradigm can be seen as ideal as it measures a pre-attentive change detection mechanism, which is present even in newborns, is sensitive to various kinds of deviances. [120]

1.Our results showed a maturation effect as we have found differences between the two age groups in case of the first time window of the phoneme detection. The younger group had bigger mismatch responses than the older group, although this difference was not significant. The distribution of the response was more marked at the right hemisphere in case of this age group. [121] We can conclude, that preterm and term infant are able to discriminate phonemes (zöngés-zöngétlen) at already as early as 6 months of age.

2.A further interesting result of our study was that no indication of a mismatch response could be found in the early time window (between 300-350 ms) of the ERPs elicited by the stress deviant, contrary to what our research group found in adult participants. We interpreted these results, that at this young age neither a short-term trace nor a long-term representation of the native language’s regularity provide a sufficient basis for detecting the lack of stress on the first syllable of the stress deviant.

The stress on the second syllable elicited a late positive MMR in the time window of 500-550 ms resembling the MMN found in adults in the Honbolygó et al. study. [91] We have found this component in both age groups, which means that infants are able to use stress information from the early months. The fact that we found a positive ERP component in infants instead of a negative one as in adults has many precedents in the developmental electrophysiological literature. [122] Most commonly the variability of positive-negative differences in 6 month-olds is related to the maturational changes during development. Another explanation suggested by [123] who emphasize the background neural processes in different cortical layers. The different background is denoted by the different wavemorphology of the positive and negative responses. While the MMN is usually a fast component, the positive MMR responses are slower with a longer deflection. [124] In a current study, Mueller et al divide infants by the polarity of their mismatch response, and matching electrophysiological responses to behavioral results, argue for the immaturity of positive MMR. [125]

Our results showed no differences in MMN responses in the phoneme deviant condition between the groups, which means a relatively intact maturation of phoneme processing of preterm infants as compared to their contemporaries. However, the mismatch responses measured in the stress condition revealed significant between group differences, which showed the developmental course of processing to the acoustically salient word stress cue. These results strengthen the view that emphasizes the intrauterine experience in case of prosodic/stress processing. Similarly Gonzales-Gomez and Nazzi argue about the role of intrauterine experience, that premature infants show developmental lag only in case of prosodic processing because of the diminished prenatal experience, which means that we would expect differences between preterm and full-term infants only in case of stress discrimination. [126] At the same time other explanations for the developmental lag of preterm infants in case of stress processing are suggested, such as by Gimenez et al. [127] They suggest that processing differences are caused by microstructural problems of the white matter even in case of preterm infants without organic deficits.

We can conclude that our most important results are the absence of change detection difference between pre- and full-term groups in the phoneme deviant condition, but its presence in the stress deviant condition. We suggest that this result provides further evidence about the different role of intrauterine experience on the maturation of processing

speech sounds and prosodic information. Furthermore, we can conclude that infants start to use stress information in differentiating words from already as early as 6 moths of age. This study shows that ERP/MMN studies are usefull in complex developmental questions, such as language processing in preterm and term infants, when other linguistic tests are not yet feasable. It also points out suttle differences of neural processing in premature infants.

7.5. IVH Study

This large retrospective study of premature infants below 32 weeks of gestation clearly showed that outcome of IVH patients born below 32 weeks of gestational age is significantly worse than outcome of preterm infants without IVH at different ages up to 5.5 years. Poorer outcomes included both significantly lower results at the psychomotor and mental scales, a higher incidence of cerebral palsy, and higher rates of visual impairment.

We could also replicate results of other studies, that show that preterm birth even without major complications such as IVH carries a high risk of impaired cognitive and motor neurodevelopmental outcome. [29] [93] In the large group of 320 controll premature infants under the age of 32 weeks of gestation, we had an incidence of over 10% CP and 15% cognitive impairment measured with the Bayley scales of infant development. This shows that this population is very sensitive to cerebral injury even when no obvious brain pathologies are present on ultrasound. In a similar study periventricular leukomalacia (PVL) was a strong and significant risk factor for subsequent IQ <70 (RR 3.4), CP (RR 13.8, 95% C.I. 4.8 – 39.5) and major disability (RR 2.6,). [75] In our study population we had 8% PVL, that we excluded from the IVH group in order to show the single effect of intraventricular bleeding on neurodevelopmental outcome. As the incidence of severe PVL decreased in recent years, diffuse white matter injury became a more common problem. Ultrasound imaging have its limitations regarding this entity. The limitation of this study is that routine MR Imaging was not avaiable for our population, to determine the effect of additional subtle pathologies only seen on MRI.

Intraventricular hemorrhage (IVH, grades 3 and 4) was also associated with increased risk of impaired outcomes, particularly CP (RR 17.6,), and major disability. [128] The strengh of our study is that lowe grades of IVH have been analyzed in detail,

showing that mild cerebral lesions in this population affect long term outcome significantly.

We had a high rate of amnion infection syndrome with 40-50% in the controll and IVH groups. These results indicate that the *in utero* exposure to inflammation and inflammatory mediators may not have a strong direct effect on risk for severe adverse neurodevelopmental outcomes, but contribute significantly to extreme premature birth. Alternatively, such exposure may have an indirect effect via a pathway that is mediated through the association of such exposure to increased risk of certain neonatal conditions such as IVH, PVL, and lung disease that, in turn, result in increased risk for a severe adverse neurodevelopmental deficit.

The analysis of the gestational age subgroups below and at or above 28 weeks showed in all parameters significantly lower results for patients below 28 weeks of gestational age compared to patients born at or above 28 weeks of gestational age. In all parameters, an increase of abnormal results with increasing grade of IVH could be observed. Even patients with IVH grade II showed high percentages of abnormal scores at 2, 3, and 5 years of age, and infants born below 28 weeks of gestational age with IVH I and II showed significantly worse results when compared infants with IVH I and II born above 28 weeks of gestational age.

The pathology of this destructive effect of even low-grade IVH could be explained by impaired cortical development. The origin of low-grade IVH is the germinal matrix located ventrolaterally to both lateral ventricles which is the source of cerebral neuronal precursors between 10-20 weeks of gestation. Later, this region provides glial precursors that migrate to cortical regions and become cerebral oligodendroglia cells or astrocytes. Destruction or absence of these cells may affect either myelination or cortical development. That explains why even low-grade IVH, especially when occurring at an early gestational age, can lead to extensive brain injury. [129] Vasileiadis et al. recently demonstrated with MRI a 16% reduction of cerebral cortical gray matter volume at term-corrected age of VLBW infants with uncomplicated IVH. [130] Another possible explanation for the high percentage of impaired psychomotor outcome is that additional pathologies, such as the high percentage of ROP (19% of all IVH patients) in this population could influence motor development and the testing situation as well.

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Our long term follow-up study clearly showed that mental and motor outcome was significantly impaired in patients born below a gestational age of 32 weeks with all grades of IVH compared to patients without IVH up to five years of corrected age. Outcome of preterm IVH infants born below 28 weeks´ gestational age was significantly poorer than outcome of preterm infants with IVH born at or above 28 weeks of gestation. There was a significant increase of developmental impairment with increasing grades of IVH. Even low-grade IVH was significantly associated with a poor mental and motor outcome in preterm infants, especially in the very preterm group (below 28 weeks´ gestational age). In this respect, more precise morphological diagnostic methods like MRI might help to detect abnormalities of the brain structure not seen by ultrasound alone which might be responsible for the surprisingly bad outcome of patients with only low-grade IVH.

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8. Conclusions

The above described studies present a variety of examples for the vulnerability of the premature/term infants nervous system. Despite joint efforts of neonatologists, obstetritians and radiologists, neurodeveloipmental impairment of premature infants remains a serious problem. Neurophysiological methods became standard procedures in everyday decisionmaking. The amplitude integrated EEG plays an essential role in continuous seizure detection, basic screening for adequate maturation and in prognosticating neurodevelopmental outcome in different pathologies, especially in neonatal encephalopathy. Flash visual evoked potentials (VEPs) have great additional value in defining severity of hydrocephalus and its impact on brain function.

The strength of the **Hydrocephalus study** is to show how accurately neurophysiological methods define brain disfunction before and after the neurosurgical intervention to decrease elevated intracranial pressures. Flash visual evoked potentials and amplitude integrated EEG changes paralelly with intracranial pressure elevation and normalisation. Reconvalescence of weve latencies of fVEP and aEEG patterns are observed and in most cases normalisation can be accurately followed. The degree of ventricular dilatation on cranial sonography has not shown significant correlation with the delay of fVEP latencies and aEEG patterns. This underlines the fact, that cranial ultrasound is just a morphologic/imaging method of the central nervous system and does not provide sufficient information about the extent of constraint on brain function. Our third hypothesis regarding later neurodevelopmental outcome and the timing of neurosurgical intervention could be not answered as of yet, due to lacking outcome data for all of the study patients.

Neuroimaging proved to be part of the everyday practice of NICUs. The increasing number of MRI studies provide information regarding the pathophysiology of different perinatal problems such as birth asphyxia or white matter injuries and play a major role in understanding normal brain development. Our **MRI-compatible Incubator Study** have shown, that MR imaging with the use of the INC is a safe and clinically informative examination even in the most unstable, critically ill premature infant. A separate analyses has shown, that patients under 2000 gram profitied mostly from this imaging device. The MR Imaging has added reliable and important diagnostic information in more than 50% of all cases and management changes were initiated also in more than half of the study

population. The safety of the imaging process increasaed, as there was no need terminate the imaging process due to instability or increase sedatives using the INC.

We were able to reproduce international results in our **Asphyxia study**, that early aEEG examination has a better correlation with neurodevelopmental outcome in patients with hypoxic-ischeamic encephalopathy, than late examination after the 1st week of life. We could also demonstrate that late MR Imaging was superior to early examination, regarding outcome prognosis. Both methods showed high sensitivity and specificity for adverse outcome. The combination of the two methods provides neonatologists with a reliable tool in clinical decision making and parent councelling, although the process of withdrawal of care in severe cases of birth asphyxia still remains a complex question, that has to be answered individually for every patient. Additional sequences to routine MRI protocolls in neonatal encephalopathy, such as proton spectroscopy should be added, as they improve the sensitivity and specificity of early MR Imaging.

In the **MMN Study** we were able to demostrate that speech maturation can be assessed with event related potentials and the MMN component in infants in the first year of life. There was only an age dependent maturation in the phoneme deviant condition (banán-panán), which demostrates that there is no difference in the detection voiceless and voice (zöngés-zöngétlen) phonemes. In contrast we found differences in speech perception between term and premature infants at 6 and 10 months of age regarding stress word detection. Premature infants had smaller MMN responses detecting atypical stress at the second syllable at both age groups, which suggests a developmental lag in language processing in premature infants. Furthermore, we can conclude that infants start to use stress information in differentiating words already at six months of age.

As the most common cause of hydrocephalus is caused by IVH in premature infants, it is extremely important to understand the extent of neurodevelopmental deficit in all grades of this pathology. The **IVH study** points out, that even in low grade IVH there is a significant risk for abnormal neurodevelopmental outcome, especially in extremely premature infants and the severity of IVH correlates with adverse neurodevelopmental outcome. We could also prove that gestational age is an independent variable in patients with IVH for neurodevelopmental outcome, as extreme premature infants under 28 weeks of gestation showed a worse outcome in all IVH groups when compared to outher preterm populations.

Minimal intraventricular bleedings leed to a maturational deficit due to the proinflamatory response and the sensitivity of olidendrocytes and subplate neurons. Cranial ultrasound has its limitations defining the extent and severity of IVH, this is why it is so important that MR imaging techniques and the MRI compatible incubator is aviable for this extremely sensitive and unstable population. Brain development can be followed by aEEG maturation, where sleep-wake cycling and background pattern are good indicators of normal neurodevelopmental outcome and intact brain function. Specific areas such as linguistic development can be studied with event related potentials, such as miss match negativity, where prosodic information and speech processing are essential components in normal speech maturation. The extent of developmental deficit in premature infants can be studied with standardised psychological tests, but evoked potentials and event related potentials provide reliable information in all pediatric age groups.

Neuroimaging prooved to be part of the everyday practice of NICUs. The increasing number of MRI studies provide information regarding the pathophysiology of different perinatal problems such as birth asphyxia or white matter injuries and play a major role in understanding normal brain development. The combination of imaging and neurophysiological methods is not only essential in everyday clinical decision making, such as neurosurgical intervention with PHVD, but also provide reliable prognostic information for neonatologists and parents as it is described in the asphyxie study.

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9. Összefoglalás

A koraszülöttek túlélési esélyei jelentősen emelkedtek az utóbbi évtizedekben, azonban a kezdeti lelkesedést beárnyékolja az a tény, hogy a túlélő koraszülöttek jelentős része valamilyen fokú fejlődésneurológiai károsodásban szenved, amelyek a korai idegrendszeri fejlődési zavarokra és kórképekre vezethetők vissza. A neonatológiai ellátásban az inkubátor melletti neurofiziológiai vizsgálatok alkalmasak az agyfunkció folyamatos követésére, míg a képalkotó vizsgálatok közül a mágneses rezonancia vizsgálat a legalkalmasabb módszer az idegrendszeri kórképek pontos meghatározására.

Eredmények: A **'hydrocephalus vizsgálat'** során kimutattuk, hogy a fVEP és az aEEG alkalmas az agynyomásfokozódás követésére hydrocephalus esetén. Az **'asphyxiás újszülöttek vizsgálata'** során kimutattuk, hogy a korai aEEG és a késői újszülöttkori MR vizsgálat szignifikáns korrelációt mutat a fejlődésneurológiai kimenetellel. Az **'MR-kompatibilis inkubátor'** vizsgálatunk alátámasztotta a neonatológiai ellátásban az MR fontosságát **'Agyvérzett koraszülöttek vizsgálata'** igazolta, hogy az agyvérzés súlyossága és a terhességi kor független rizikófaktora a fejlődésneurológiai kimenetelnek. Kimutattuk az **'MMN vizsgálattal'**, hogy a csecsemőkori nyelvi fejlődés elemzésére az eseményhez kötött potenciálok jól alkalmazhatók.

Tudományos munkámat összefoglalva kijelenthető, hogy a neurofiziológiai vizsgálatoknak- aEEG és fVEP- jelentős szerepe van a koraszülött idegrendszeri kórképek követésében és a terápia kialakításában. Alkalmasak az agy aktuális funkciójának megítélésére. A képalkotó eljárások, elsősorban a koponya MR-vizsgálat a koraszülöttekben egyre inkább nélkülözhetetlen a pontos diagnózis felállításában. A neurofiziológiai módszerek önmagukban, illetve a képalkotó eljárásokkal kombinálva alkalmasak a fejlődésneurológiai prognózis megbízható kialakítására, a kognitív fejlődés vizsgálatára és aktuális terápiás döntéshozatalra.

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10. Summary

The survival rates of premature infants have increased significantly in recent decades, but the early optimism is dimmed by the fact, that a large propotion of these infants end up with developmental disabilities, based on early brain insults and pathological brain maturation. Neurophysiological methods are usefull in following brain function in extreme premature infants even in the incubator, while magnetic resonance imaging proved to be superior in neuroimaging and in the diagnostics of pathological brain leasions of this fragile population.

The aim of this thesis is, through 5 clinical studies presentingt the role of neurophysiological and neuroimaging methods in monitoring and diagnosing neuropathological events in premature infants and to show their usefulness in prognosticating neurodevelopmental outcome.

Results: The hydrocephalus study proved, that flash Visual Evoked Potentials and amplitude integrated EEG are usefull methods in the monitoring of ventricular dilatation in posthyaemorrhagic and congenital hydrocephalus. In the asphyxia study we have shown, that early aEEG and late MR examination are significantly correlated with neurophysiological outcome. The MR-compatible incubator study proved the importance of MR examinations in the extreme premature infant. The IVH study have shown that the severity of the IVH and the gestational age are independent risk factors of neurodevelopmental outcome in premature infants. The MMN study proved that event related potentials are usefull in the analysis of language development in infants.

Summarising my work we can conclude that neurophysiological methods, especially aEEG and fVEP, play an essential role in the diagnosis and therapy of brain leasions in premature infants. They are suitable for brain function analysis. Imaging processes, primarily MR examinations are essential in the exact diagnosis. Optimising the imaging process benefits especially the most fragile extreme premature infant. Neurophysiological methods alone, or in combinations with neuroimaging are suitable for providing reliable prognosis, study cognitive development and help everyday therapeutic decision making in the neonatal population.

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12. Bibliography of the candidate's publications

12.1. Related Publications

1. Róna, Z., Klebermass K, Cardona F, Czaba CD, Brugger PC, Weninger M, Pollak A, Prayer D, (2010)*Comparison of neonatal MRI examinations with and without an MR-compatible incb ator: Advantages in examination feasibility and clinical decision-making.* Eur J Paediatr Neurol,. 14(5): p. 410-17. IF: 1,994

2. Klebermass-Schrehof, K., Czaba C, Olischar M, Fuiko R, Waldhoer T, Rona Z, Pollak A, Weninger M. (2012) *Impact of low-grade intraventricular hemorrhage on long-term neurodevelopmental outcome in preterm infants.* Childs Nerv Syst,. 28(12): p. 2085-2092. IF: 3,045

3. Klebermass-Schrehof, K., Róna Z, Waldhör T, Czaba C, Beke A, Weninger M, Olischar M, (2012) *Can neurophysiological assessment improve timing of intervention in posthaemorrhagic ventricular dilatation?* Arch DisChild Fetal Neonatal Ed,IF:1,542

4. Rago A. Honbolygo F. Rona Z. Beke A Csépe V (2014) Effect of maturation of suprasegmenal speech processing in full- and preterm infants: a mismatch negativity study, Res. Dev Disabil, 35(1): 192-202 IF:2,483

12.2. Unrelated Publications

1.Müller K, Róna Zs, Farkas V (2005) *Startle betegség serdülőkorú leánybetegen* Gyermekgyógyászat,56(5): 547-55.

2. Harmath Á; HAjdú J. Hauzmann E. Pete. B. Rona Zs. Papp Z. (2007) *Experiences in the perinatal management of congenital diaphragmatic hernia during the last 15 years in a tertiary referral institute* Fetal Diagn Ther, 22: 209-16. IF: 0,844

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