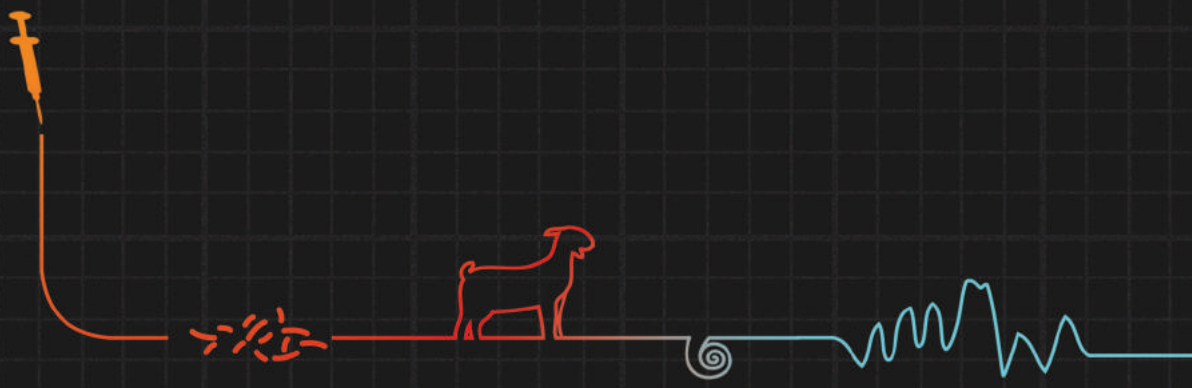

PERINATAL FACTORS AND HEARING OUTCOME

Diane Smit

Perinatal factors and hearing outcome

Adriana Leni Smit

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Perinatal factors and hearing outcome

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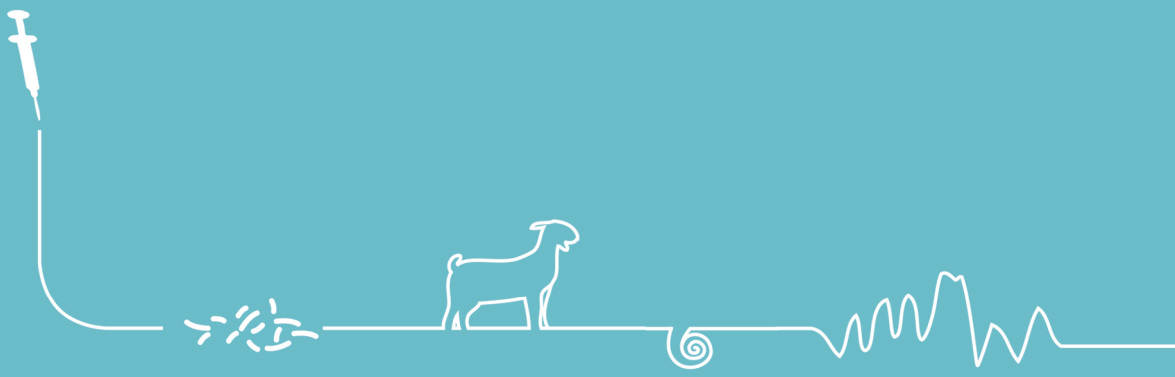
Contents



CHAPTER 1

Introduction and outline of the thesis

Introduction

Neurodevelopmental outcome and hearing loss

Although the survival of preterm infants over the last decade has been improved by advances in perinatal care, the prevalence of major neonatal and long-term morbidities in this group has not significantly changed.[1] The developing premature central nervous system is highly vulnerable to intrauterine and perinatal events[2] and a large number of very preterm infants survive with long-term neurodevelopmental impairments.[3] Neurodevelopmental disabilities, defined as chronic disorders of the central nervous system function due to malformation or injury of the developing brain, contains a spectrum of disorders. The more subtle disorders include language disorders, learning disabilities, attention deficit-hyperactivity disorder (ADHD), minor neuromotor dysfunction or developmental coordination disorders, behavioral problems, and social-emotional difficulties. The group of major disabilities includes cerebral palsy (CP), mental retardation and sensory impairments as well as visual or hearing loss.

Hearing loss; a major childhood disability

The auditory system encompasses different parts; from the cochlea, sound information travels down the auditory nerve, through intermediate stations such as the cochlear nuclei and superior olivary complex of the brainstem and the inferior colliculus of the midbrain. The information eventually reaches the thalamus, and from there it is projected to the primary auditory cortex in the temporal lobe (Figure 1).

When the integrity of this system is affected in children, this not only results in hearing loss, but also disrupts acquisition of spoken language and other domains of neurocognitive development, e.g. cognitive, social and academic functioning.[4, 5] The incidence of congenital hearing loss amounts 1–3 per 1000 amongst full-term live births in the Western world [6–9] compared to 2–4 per 100 live births in preterm infants.[7] However, numbers vary in relation to used definitions of severity and laterality. Besides this, by the interaction between environmental and genetic factors the prevalence and the etiology of hearing loss can differ in various parts of the world and within different socio-ethnic groups.[10]

Risk factors of hearing loss in children

Taking this in account, several indicators are generally considered as a risk factor for hearing loss in childhood and listed as such in ‘principles and guidelines for early hearing detection and intervention programs’ by the Joint Committee on Infant Hearing.[11] Within this guideline several variables as neurodegenerative disorders, syndromes, intrauterine- and postnatal infections and neonatal parameters such as hyperbilirubinemia and assisted ventilation are mentioned as risk factor. However, the significance of other factors within the onset of hearing loss like lower birth weight, low Apgar scores, and interactions between these entities are still under debate.[10]

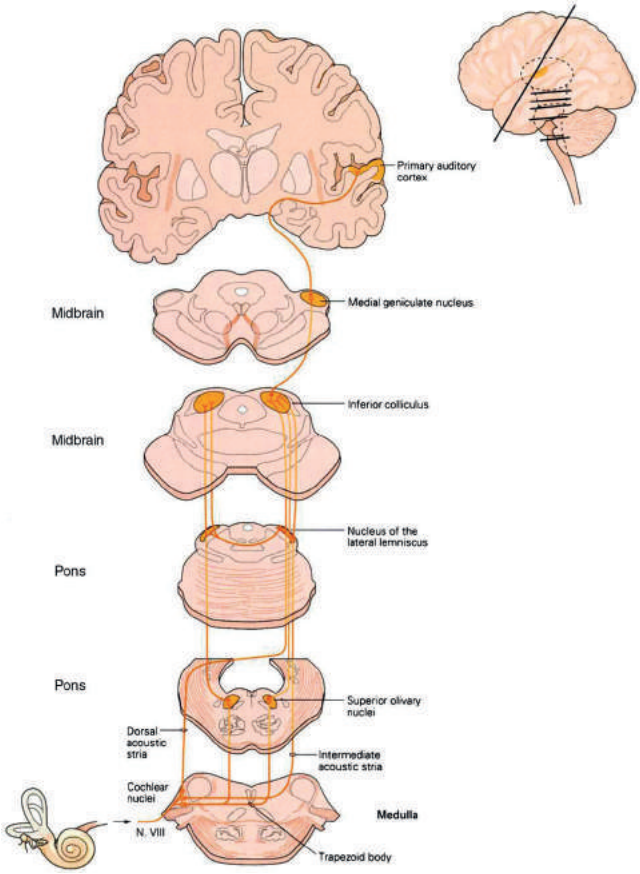


Figure 1. The central auditory pathway. Adjusted from *Kandel ER. Principles of Neural Science, Fourth Edition. New York: McGraw Hill; 2000:604.*

Perinatal asphyxia and hearing outcome

To be mentioned in more detail in relation to hearing outcome, perinatal asphyxia is a clinical syndrome at birth characterized by an impairment of exchange of the respiratory gases (oxygen and carbon dioxide) resulting in hypoxemia and hypercapnia, accompanied by metabolic acidosis.[12] In developed countries, perinatal asphyxia affects 1–6 newborns per 1000 live term births.[13] Perinatal asphyxia is one of the major risk factors for severe neurological sequelae in newborn infants.[14] Recent randomized controlled multicenter trials have shown that in more than 60 % of term or near term infants with perinatal asphyxia either died or suffered moderate to severe long-term impairments such as cerebral palsy and learning disabilities.[15, 16] Hearing loss has been reported in up to 17 % of infants with a history of perinatal asphyxia.[17] Several clinical studies suggest that there are already direct negative effects on hearing parameters within several hours to days after a severe event

of peri- or postnatal hypoxia-ischemia.[18–20] However, studies evaluating hearing outcome in relation to perinatal respiratory and circulatory parameters are not consistent. In a Dutch cohort of neonatal intensive care unit (NICU) infants born with a gestational age <30 weeks and/or a birth weight <1000g, a positive correlation was found between severe birth asphyxia (Apgar scores <5 at 1 min or <7 at 5 min) and adverse neonatal hearing outcome by automated auditory brainstem response (AABR) screening (OR 1.7, 95 % CI 1.0–2.7).[21] In a similar analysis under NICU graduates by Van Dommelen et al. Apgar score after 5 min was related to hearing loss identified by univariate analyses, but statistical significance disappeared after correcting for other factors.[22] This can be explained by the dependency between the variables Apgar score and other parameters at the same time. This outcome illustrates that the identification of a specific variable related to perinatal asphyxia and their role in the onset of congenital hearing loss is complicated by the studied (confounding) factors as well as characteristics (e.g. gestation) of the studied population. Besides this, sample size, definitions of variables like perinatal asphyxia and the severity of the perinatal hypoxic-ischemic event will influence this outcome.

Chorioamnionitis; a risk factor for congenital hearing outcome ?

Besides perinatal asphyxia, other factors as chorioamnionitis are strongly associated with perinatal outcome. A chorioamnionitis is a status of intrauterine inflammation/infection of either mixed fetal-maternal (choriodecidual space) or fetal origin (chorioamniotic membranes, umbilical cord and amniotic fluid) (Figure 2).[23] Histological studies have shown that a chorioamnionitis is present in ~10 % of term infants, in about 50 % of very low birth weights infants (VLBW) and up to 80 % of the extremely low birth weight infants (ELBW).[24] As most pathogens associated with chorioamnionitis are of low virulence, in about 65 % the inflammatory process remains subclinical.[25, 26]

In clinical studies histological chorioamnionitis is associated with an increased risk for white matter disease including periventricular leukomalacia (PVL) and cerebral palsy (CP) in both premature and term infants.[2, 28–30] Moreover, several studies reported a relationship with neurodevelopmental delay after birth,[2, 31–35] although others have been unable to confirm this.[36] These inconsistent data can be explained by the fact that chorioamnionitis represents a spectrum from severe fetal inflammatory response syndrome with funisitis to a mild mainly maternal disease with little effect on the fetus.[37] Little is known about the effect of chorioamnionitis on the peripheral and central auditory system. While a study by Suppiej et al. demonstrated a relationship between preterm histological chorioamnionitis and hearing loss during childhood,[38] other studies failed to reproduce these findings.[39–41]

Experimental animal models to investigate effects to the auditory system

As conclusions about the role of a specific perinatal factor in the onset of congenital hearing loss is complicated by definitions, confounding factors and limitations of the studied

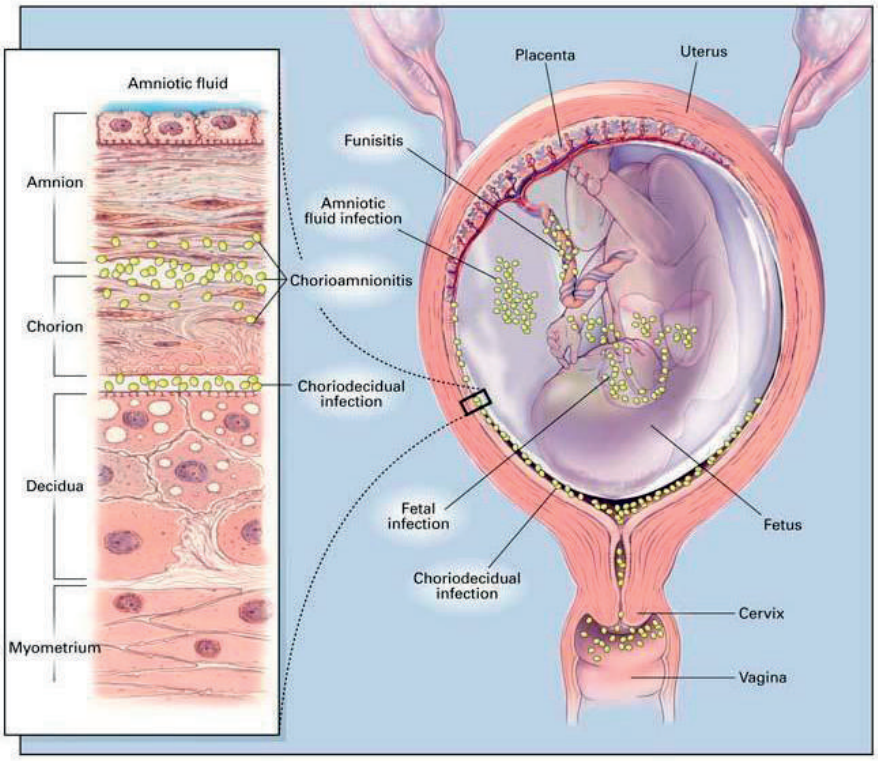


Figure 2. Potential sites of bacterial infections within the uterus. *Reproduced with permission from Goldenberg et al.,[27] Copyright Massachusetts Medical Society*.

clinical cohort, several models have been used to study effects to the peripheral and central auditory system. Experimental animal models have been developed to analyze the role of maternal inflammation on the perinatal brain.[42] While infections can be modeled by injection of live bacteria into animals, the mortality associated with true bacterial sepsis is high. Experimental animal models using isolated components of bacterial cell wall, such as lipopolysaccharides (LPS), permits a higher rate of survival and initiate many of the events associated with systemic infection. The chronically instrumented fetal ovine model is widely used to observe neonatal outcome under a range of stimuli like LPS exposure[43–45] and hypoxia-ischemia.[46, 47] In fetal sheep, the neurodevelopment is very similar to the human situation[48] and frequency range of hearing is comparable between sheep (approximately 100 Hz – 30 kHz) and humans (64 Hz – 23 kHz).[49] The possibility of applying this ovine model to a broader set of questions concerning the fetal and neonatal hearing is of great interest to elucidate the relationship between perinatal factors and early hearing outcome in a unique way.[50, 51]

Role of imaging techniques in evaluation of the inner ear

Besides audiometric and electrophysiological measurements, imaging techniques have been used to assess the integrity of the inner ear and central auditory pathway. MR imaging has been proven to be of additional value to CT scanning in evaluating the patency of the cochlea in a process of inner ear inflammation.[52] So far, there is very little information about the dynamics of the inflammatory responses of the inner ear in which several stages can be identified e.g. acute inflammation, fibrosis and ossification.[53] Floc'h et al. recently demonstrated the use of high field MR imaging in a guinea pig model of inner ear inflammation.[54] By this a quantitatively and qualitatively assessment was performed of several key elements of the inflammatory response with signs of transient enhanced cochlear vascular permeability and indications of infiltration of phagocytic cells into the cochlea. A better understanding of this process of cochlear inflammation can lead to the development of targeted treatments to reduce the deleterious effects of inflammation on the delicate structures of the inner ear. By the introduction of ultra high resolution MR imaging of the inner ear[52, 55] and the application of MR imaging even in fetal conditions [56, 57] new possibilities have been created to evaluate the inner ear in relation to several factors. However, the feasibility of these MR techniques to this perspective still needs further study.

Aims and outline of this thesis

Although evidence is increasing that perinatal events are involved in the pathophysiology of hearing loss, many questions about the role of perinatal factors in the onset of hearing loss remain unanswered. Therefore, the main purpose of this thesis is to provide insight into the contribution of individual perinatal factors to hearing difficulties after birth.

The following questions were addressed:

- Is there a potential role for LPS in congenital sensorineural hearing loss (SNHL) according to the literature? In Chapter 2, we provide a review of the current literature to elucidate the potential role of this toxin in congenital SNHL and to identify the pathogenesis and potential transmission routes in utero to the auditory system.
- Does an intrauterine LPS induced chorioamnionitis affect the auditory system? In Chapter 3, we will introduce an experimental animal model to study hearing outcome in relation to intrauterine and perinatal events. To answer the question, this animal model is used to evaluate the relation between intrauterine LPS induced chorioamnionitis and fetal perilymphatic inflammation as well as hearing outcome after birth by auditory brainstem response measurements.

- Is histological chorioamnionitis associated with an adverse neonatal hearing outcome? For this purpose, two cohorts of very preterm human newborns were linked to placental histology and hearing screening outcome by AABR in Chapter 4. By this, predictors of abnormal hearing screening outcome could be identified within this population.
- What is the functional effect of perinatal asphyxia and perinatal propofol anesthesia to hearing parameters? In an attempt to answer this question, the lamb model was used to induce perinatal asphyxia by umbilical cord occlusion and analyze the effect to ABR measurements after birth in relation to anesthetic treatment in Chapter 5.
- Is the application of 7TMR imaging feasible to study perinatal effects to the inner ear? To test the feasibility of 7T MR, the temporal bones of ex vivo preterm lambs were assessed to evaluate the microstructures of the inner ear in Chapter 6.

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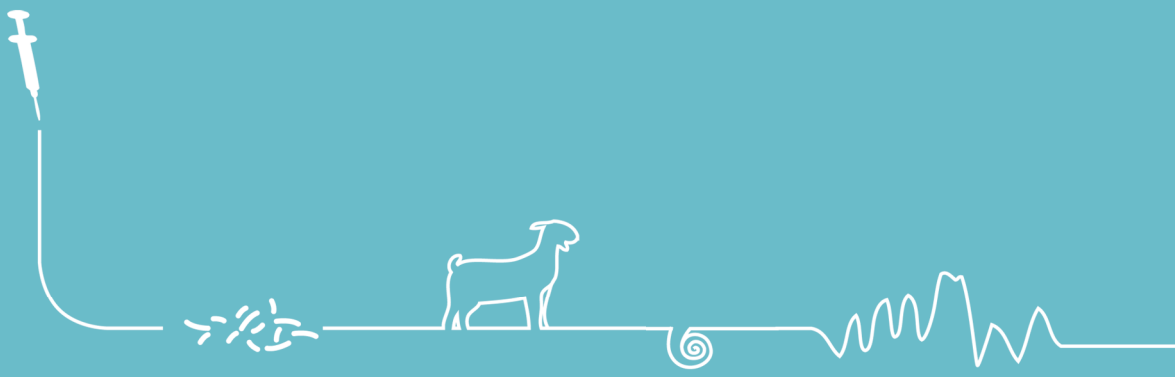

CHAPTER 2

Potential role for lipopolysaccharide in congenital sensorineural hearing loss

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S.G.H. Litjens
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Journal of Medical Microbiology. 2010;59(4):377-83

Abstract

Congenital sensorineural hearing loss (SNHL) is common. In the Western world, the incidence is 1–3 per 1000 live births. The aetiology encompasses genetic and non-genetic factors accounting for 55 % and 45 % of cases, respectively. Reports that describe the contribution of intrauterine infection to the occurrence of congenital SNHL are limited, and comparative analysis of the different pathogens is lacking. Lipopolysaccharide (LPS), a product of bacteriolysis, has been demonstrated to be associated with inner ear damage in experimental studies. To elucidate the potential role of this toxin in congenital SNHL and to identify the pathogenesis and transmission routes, we reviewed the literature. We speculate that different routes of exposure to LPS *in utero* may result in congenital inner ear damage.

Introduction

Congenital sensorineural hearing loss (SNHL) in childhood is common. In the Western world, the incidence amongst full-term live births amounts to 1–3 per 1000.[1–4] In preterm infants, the incidence is even higher, with 2–4 per 100 live births.[4] The aetiology of congenital SNHL is attributable to genetic and non-genetic factors in 55 % and 45 % of cases, respectively.[1] Non-genetic causes are divers, e.g. perinatal exposure to hypoxia,[5] acidosis,[6] otoxicity of drugs such as aminoglycoside or loop diuretics[7] and viral or bacterial infections.[8] In this review, we will focus on the infectious causes of congenital SNHL, of which infection with cytomegalovirus (CMV) is the most common.[1] The estimated prevalence of CMV infection amounts to 0.2–2.2 % of all live births.[9, 10] Ten to twenty per cent of infants congenitally infected with CMV had varying degrees of hearing loss at birth.[11] Besides CMV, other micro-organisms, e.g. *Toxoplasma gondii,* measles virus, mumps virus, varicella-zoster virus, parvovirus B19, herpes simplex virus, *Treponema pallidum* and rubella virus, have been identified to cause unilateral or bilateral congenital SNHL, which may be temporary or permanent, with or without loss of vestibular function.[8, 12]

Bacterial products leading to SNHL are the subject of increasing interest. Lipopolysaccharides (LPS) is released from the outer wall of Gram-negative bacteria such as *Escherichia coli* and *Neisseria* spp. after bacteriolysis.[13] This bacterial endotoxin consists of a lipid A, a core polysaccharide and an O antigen and is known for its multiple effects on biological systems, e.g. induction of inflammation and the modulation of the immune responses.[13] It is also associated with functional and morphological inner ear damage in experimental animal models,[14–16] which we will discuss in the next paragraph. We hypothesize that this toxin, by means of an intrauterine infection, can play a role in the pathogenesis of congenital SNHL.

LPS-induced functional and morphological inner ear changes

By discovering LPS in middle ear effusions in chronic otitis media,[17–19] attention was drawn to its crucial role in the onset of SNHL. Subsequent functional studies did demonstrate an elevation of hearing level thresholds after LPS exposure. Comis *et al* and Tarlow *et al.* revealed early onset SNHL within 2 h after LPS application into the guinea pig inner ear by cochlear potential measurement.[20, 21] In a similar model, high frequency losses were observed by auditory brainstem response measurement within 1 [16] or 2 [13] days after LPS application. A gradual improvement of responses was seen after a 5 day interval to near baseline level by 28 days.[13] To explain the LPS-related functional impairment of the inner ear, several morphological temporal bone studies were designed using different animal models. These findings are summarixed in Table 1. Comis *et al*. and Tarlow *et al*.

Table 1. Effects of LPS on the inner ear in experimental animal models.

Reference	Animal model	LPS origin	Methods*	Evaluation time after application	Results†
Nakai *et al*. (1980)	Mice	*E. coli*	Repeated intradermal and intravenous injection	3 days / 1.5 months / 3 months	Degeneration of OHCs & IHCs and stria vascularis. Vacuolization and degeneration of vestibular sensory cells; vacuolization of dark cells.
Spandow *et al*. (1989)	Rat	*E.coli*	Instillation into RW niche	2 weeks	OHC loss
Kawauchi *et al*. (1989)	Chinchilla	*Salmonella typhimurium*	Instillation into tympanic cavity	12–72 h	Bleeding and inflammatory cell recruitment in perilymphatic space including the spiral ligament, strial swelling and sensory cell degeneration
Lim *et al*. (1990)	Chinchilla	*Salmonella typhimurium*	Instillation into tympanic cavitiy	12 h-3 weeks	Inflammatory infiltrates in scala tympani, vestibule and spiral ligament, strial swelling, and sensory cell degeneration
Comis *et al*. (1991) Tarlow *et al*. (1991)	Guinea pig	*E.coli*	Perfusion in scala tympani or injection in CSF	hours	Damage cochlear hair cells, swelling of the tectorial membrane
Darrow *et al*. (1992)	Guinea pig	*Salmonella typhimurium*	Inoculation in scala tympani	1–7 days	Inflammatory infiltrates in perilymphatic spaces
Guo *et al*. (1994)	Guinea pig	*E. coli*	Perfusion into perilymphatic space via RWM	1/3/5 days	Damaged stria vascularis
Watanabe *et al*. (1995)	Guinea pig	*E.coli*	Intraperitoneal injection and application at RW niche	2 days / 1 day	Inflammatory cells in endo- , perilymphatic spaces, stria vascularis, spiral ligament and organ of Corti. Widened intercellular spaces in the stria vascularis. Bleeding in the endo- and perilymphatic spaces.
Watanabe *et al*. (2001)	Guinea pig	*Not specified*	Transtympanical injection	2 days	MPO in the spiral ligament, stria vascularis and supporting cells of the organ of Corti
Watanabe *et al*. (2002)	Guinea pig	*E. coli*	Transtympanical injection	2 days	MPO in the sensory epithelium and dark cell area

*CSF, cerebrospinal fluid; RW: round window, RWM: round window membrane.

†IHCs, inner hair cells; OHCs, outer hair cells; MPO, myeloperoxidase.

showed loss of hair bundles of inner and outer hair cells as well as swelling of the tectorial membrane directly after LPS application into the inner ear of guinea pig.[20, 21] Darrow *et al*. demonstrated in the same species a dosage-dependent inflammatory reaction with inflammatory cells in the perilymphatic spaces; lower LPS dosages resulted in minimal inflammation during the evaluation period of 7 days.[13] Higher dosages showed a higher degree of inflammation after 2–4 days which resolved at day 7.[13] Also the stria vascularis revealed LPS-related alterations in the first days after exposure.[16] Watanabe *et al.* demonstrated similar results after LPS application into the middle ear.[22] Moreover, neutrophil recruitment was seen by by myeloperoxidase staining in multiple cochlear structures 48 h after LPS exposure.[14]

Other experimental animal models and application schemes demonstrated analogous patterns of inflammatory and structural changes within small variations of time of onset. Within 12–72 h post middle ear application of LPS in a chinchilla model, bleeding and inflammation in the perilymphatic spaces and spiral ligament, strial swelling and sensory cell degeneration were observed.[23, 24] Repeated application of LPS at the round window niche of rats by Spandow *et al*. resulted in hair cell loss within 2 weeks.[25] Nakai *et al*. observed similar results as well as a total disappearance of outer hair cells and nerve fibers in the spiral lamina after a period of 1.5 months.[26]

Pathogenesis of LPS-induced inner ear dysfunction

LPS is known for its diverse effects on the inflammatory cascade through activation of the Toll-like receptor 4 (TLR-4). These receptors are expressed in the inner ear by immune cells such as macrophages and B cells, and non-immune cells, including fibroblasts and epithelial cells.[27] TLR-4 signalling activates nuclear factor kappa B, which is the 'master switch' for inflammation-mediated cytokine production and release of mediators such as nitric oxide (NO).[25, 28–30] In the resulting network of pro- and anti-inflammatory cytokines, interleukin-1 (IL-1), IL-2, tumour necrosis factor alpha and transforming growth factor beta are the most important cytokines and modulators for the inflammatory response as well as for homeostasis and tissue repair.[31, 32] Recent studies provided new insights into the relationship between LPS and NO related potential inner ear damage.[33] NO has a role in regulation of vascular tone, auditory transduction and cochlear and vestibular neurotransmission.[34–39] Besides this, NO has a dose-dependent action. In small amounts, it has a defensive action against invading organisms by inhibition of adhesion molecule expression, cytokine and chemokine synthesis and leukocyte adhesion and transmigration.[40–42] When produced in larger amounts, NO can be injurious to healthy tissue such as cochlear cells[42–48] due to formation of peroxynitrate (ONOO-) by reaction of NO with superoxide anion (O2-).[49] (Fig. 1)

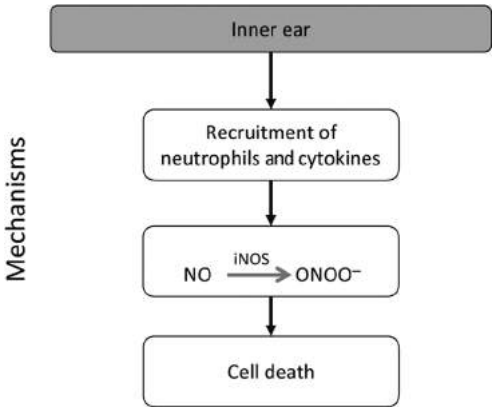

Figure 1. Mechanisms leading to an LPS-related inflammatory response of the inner ear and cell death through the action of cytokines and NO.

NO is synthesized by NO synthase (NOS). This enzyme has different isoforms: constitutive and inducible NOS (cNOS and iNOS).[39] The neuronal cNOS is always present and produces, depending on the intracellular calcium levels, small amounts of NO. In contrast, the iNOS isoform is usually absent, but can be synthesized by virtually any cell type when adequately stimulated by cytokines or LPS.[14, 28, 39, 42, 50–52] This LPS-mediated elevation of iNOS was also demonstrated in the walls of blood vessels of the cochlear lateral wall and in different cells of the organ of Corti, including in stereocilia of inner and outer hair cells, pillar cells, hair cell nerve fibers and in marginal cells.[33, 50, 51] By this mechanism, the formation of NO and subsequent peroxynitrite could be responsible for the described functional and morphological cochlear alterations after LPS exposure of the inner ear.

In utero lesion of the inner ear

As discussed above, experimental studies have shown LPS-induced functional and structural inflammatory changes in the inner ear. However, several pathways must be considered to explain LPS-related *in utero* inflammatory changes of the inner ear and subsequent potential congenital SNHL.

Fetal inflammation is the consequence of a chorioamnionitis.[53] This is defined as an inflammation of the amniotic fluid and membranes.[54] This inflammatory process is generally regarded as a continuum with early-stage maternal neutrophils involved progressing to later stages with a fetal neutrophil inflammatory reaction.[54] Micro-organisms responsible for chorioamnionitis can reach the chorioamnion by the abdominal cavity through the Fallopian tubes or inadvertent needle contamination at the time of amniocentesis or chorionic-villus sampling, by passage through the cervix from the vagina,[54, 55] or transplacentally due to a

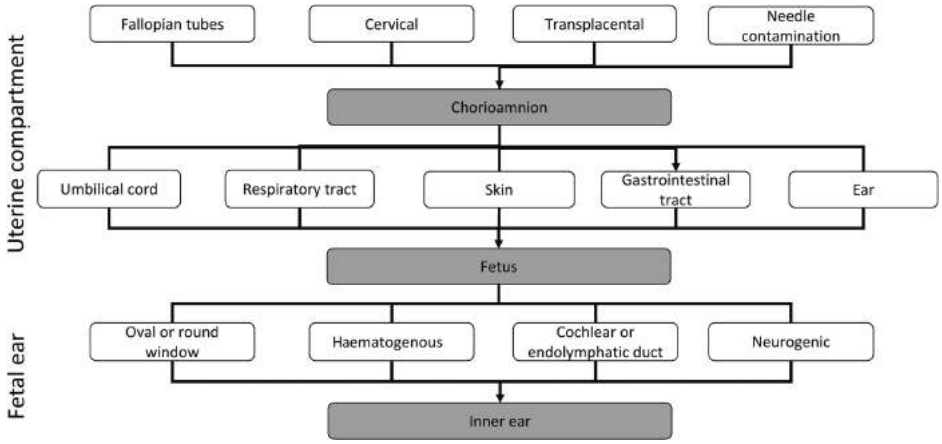

Figure 2. Inflammatory pathways from the uterine compartment to the fetal inner ear.

maternal systemic infection (Fig. 2).[54] The micro-organisms or the microbial products, once present in the amniotic fluid, can gain access to the fetus [54] and evoke a local inflammatory response by entry via the respiratory tract, skin, gastrointestinal tract and ear.[56] When neutrophils infiltrate the umbilical cord vessels,[57] this can be considered a progression of a chorioamnionitis to a funisitis, which results in a fetal systemic inflammatory response syndrome.[56, 58] This is associated with increased morbidity and mortality of the fetus.[53, 59]

Once the inflammatory mediators or micro-organism enter the fetus, several routes are possible for reaching the labyrinth (Fig. 2). The upper respiratory tract as well as passage of the tympanic membrane gives entry to the middle ear. Subsequently, the oval and round window are a porte d'entrée to the inner ear. The round window membrane can be passed easily by small molecular mass substances [24, 60–64] in contrast to large molecular mass substances such as albumin or LPS.[60, 65, 66] However, previous studies have demonstrated passage of the toxin and cochlear inflammation by a suspected LPS-related alteration of the membrane permeability or structure.[13, 25, 34, 35, 60–63, 67–73] Another option is a haematogenous route via blood vessels of the inner ear,[15] which are mainly located in the spiral limbus and the lateral cochlear wall.[15, 49, 74] Previous findings after LPS exposure-related damage in the endolymphatic compartment suggested blood–labyrinth barrier disruption by the inflammatory molecules and the capability of reaching the endolymph.[74] This barrier may be even more permeable at very early stages of development as shown in a rat model.[75] Also the permeability to proteins of the blood-brain barrier is affected by LPS.[27] This can result in a third, neurogenic, route, in which the infection could travel via meninges and along the eight cranial nerve through the modiolus to the spiral ganglion and limbus.[74, 76] Besides this, propagation of infections from the cerebrospinal fluid to the peri- and endolymphatic space via the cochlear aquaduct or endolymphatic duct, respectively, has been observed.[74, 76].

Role of LPS in congenital hearing loss

Regarding the described LPS-related functional and morphological effects on the inner ear as well as the presumed transmission routes, an association between an LPS-induced chorioamnionitis and congenital inner ear changes or congenital SNHL seems plausible. This is supported by the findings of Suppiej *et al.*, who recently described a positive association between a histological identified chorioamnionitis and SNHL in childhood after preterm delivery before 32 weeks gestational age.[77] They postulated a relationship with worse neurological and cochlear development and maturation.

However, it was not possible to determine whether preterm histological chorioamnionitis in itself or the higher exposure to other risk factors such as neonatal hypotension had concomitant roles in this relationship with SNHL.

The presented studies demonstrating LPS-induced functional or morphological inner ear changes were done using different experimental animal models and time intervals. To what extent these changes can be extrapolated to the human fetus has not been addressed so far. It is unclear whether the described alterations are persistent or fluctuating in time or will recover in the long term with or without removal of the stimulus. Moreover, the ototoxic potential of LPS seems to be dependent on dosage, LPS type[13] and application method. Intramuscular,[78] intraperitoneal[79] and intravascular administration[80] show more severe effects on the fetus [78, 81] than intra-amniotic LPS injection.[82]

Previous studies in a sheep model did confirm the hypothesis of mechanisms of maturation of the fetal immune system in the case of repeated exposure to LPS.[83–87] To what extent this will influence the immunological response of the fetal inner ear to prolonged *in utero* exposure to LPS is not clear. By designing a new experimental animal model we hope to answer some of these fundamental questions in the near future to elucidate the role of LPS in and mechanisms of LPS-related congenital SNHL after intrauterine fetal exposure. A better understanding of the fetal health risks can play a role in maternal screening programmes and could be a step forward in the design of preventive strategies for congenital SNHL or early clinical screening.

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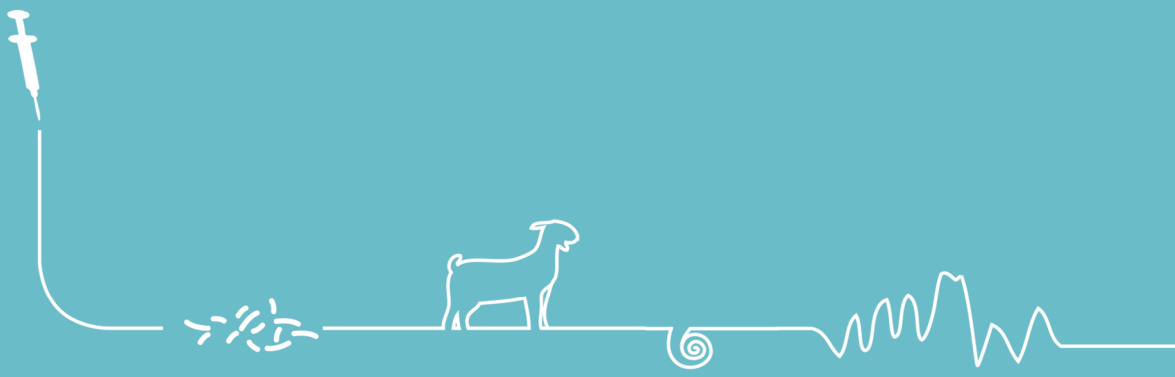

CHAPTER 3

Intrauterine lipopolysaccharide-induced chorioamnionitis in a sheep: does it affect the auditory system ?

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Abstract

Background: Fetal exposure to in utero inflammation such as chorioamnionitis is related to central nervous system injury. We hypothesized that chorioamnionitis can provoke inflammatory changes in the perilymph and alter hearing outcome.

Methods: Pregnant ewes were randomized into 2 groups: intrauterine injection with lipopolysaccharide (LPS; n = 19) or saline (n = 21). In the first experiment, fetal perilymph samples were taken for cytokine analysis. In the second experiment, consecutive bone-conducted auditory brainstem responses were obtained from 1 to 7 months after birth.

Results: Perilymph samples showed a significant elevation in interleukin 8 in the LPS group. Auditory brainstem response analysis demonstrated higher response thresholds and a prolongation of absolute peak V and interpeak intervals I to V and III to V in the LPS group compared to sham treatment.

Conclusion: Our study confirms the hypothesis that an intrauterine inflammation by LPS can result in a fetal perilymphatic inflammatory response and functional impaired hearing outcomes after birth in a sheep model.

Introduction

Chorioamnionitis is an intrauterine inflammatory process that is known to affect several organ systems in the fetus.[1] It has been associated with an increased risk for white matter disease including periventricular leukomalacia (PVL) and cerebral palsy (CP).[1–4] Moreover, several studies reported a relationship with neurodevelopmental delay after birth,[2, 5–9] although others have been unable to confirm this.[10]

Little is known about the effect of chorioamnionitis on the peripheral and central auditory system.[11] Although a study by Suppiej et al. demonstrated a relationship between preterm histological chorioamnionitis and hearing loss during childhood,[12] other studies failed to reproduce these findings.[13–15]

We hypothesized that chorioamnionitis could be responsible for an inflammatory response of the fetal inner ear and impaired hearing outcome after birth. In fetal sheep, the neurodevelopment is very similar to the human situation[16] and frequency range of hearing is comparable between sheep (approximately 100 Hz – 30 kHz) and humans (64 Hz - 23 kHz).[17] We therefore used a sheep model of chorioamnionitis induced by intrauterine exposure to lipopolysaccharide (LPS), a product from the outer wall of gram-negative bacteria.[18–22] This model has proven to induce a systemic fetal inflammatory response and brain damage with impaired electroencephalographic outcome.[18, 22, 23]

To test the hypothesis, we analyzed the inflammatory cytokine reaction in perilymphatic fluid in utero after intrauterine injection of LPS. Auditory brainstem responses (ABRs) from 1 month after birth until adolescence at 7 months were analyzed to investigate eventual onset and progression of hearing loss as well as effects of maturation of the ABR. These measurements were assessed in terms of response thresholds, absolute peak latencies, and interpeak intervals.

Material and methods

Animals

The time-mated Texel ewes and the fetuses (singletons and twins) of both genders were treated according to the guidelines of the Animal Care Committee of the University of Maastricht that approved the protocol. Ewes were randomized to ultrasound-guided intra-amniotic injection with LPS endotoxin (n = 19; 5 mg/ml, 2 ml injection, solubilized in saline and filtered; Sigma Aldrich, Saint Louis, MO, USA) or sham treatment with the same amount of 0.9 % saline (n = 21). The dosage of LPS was based on a study demonstrating that this concentration causes a chorioamnionitis as well as a systemic fetal inflammatory response.[24] Injection took place at 111 days gestational age (GA; term 147 days). Two experiments were conducted: 1) perilymph sampling (n = 11) and 2) ABR recordings (n = 29).

Experiment 1: perilymph sampling

In the first experiment, lambs from both groups (LPS: n = 5, sham: n = 6) were delivered prematurely by cesarean section at 125 days GA (comparable to 27 weeks of human gestation). Lambs received a lethal intravenous injection of phenobarbiturate immediately after delivery. The oval window was assessed directly post-mortem with a microscope through the external ear canal and tympanic cavity after removal of the brain. The stapes footplate was dried by suction and then perforated to aspire the perilymphatic fluid by a microinjector/withdrawer with a sterile fine-tipped capillary tube and stored at -80°C until assay. Perilymph samples were diluted initially 1:2 with pyrogen-free distilled water. Perilymph cytokine concentration of interleukin-6 (IL-6), IL-8, and soluble intercellular adhesion molecule (sICAM) were measured using ELISA kits (R&D Systems, Minneapolis, Minnesota), with sensitivities of 0.7 pg/ml, 7.5 pg/ml and 0.254 ng/ml, respectively. Samples were run in duplicate, and mean values were reported.

Experiment 2: ABR Recordings

In the second experiment, ABR measurements were obtained. An ABR is generated by cochlear action potentials, the auditory nerve, and subsequent structures within the auditory brainstem pathways after an acoustic stimulus. By this, recording of ABR threshold and evaluating absolute peak latencies of individual wave components and interpeak intervals provide insight in the degree of hearing loss as well as information about the integrity of the auditory pathway.

For this experiment, lambs from both groups (LPS: n = 14, sham: n = 15) were born by spontaneous delivery at term GA (mean: 147 days, range: 141–150 days). To obtain longitudinal ABR measurements, the lambs were kept in a natural environment and fed at libitum for 7 months after which a lethal dose of intravenous T61 (Veterinaria AG, Zürich, Switzerland) was administered. To control for eventual maturational aspects of ABRs as well as onset and/or progression of hearing loss, ABR recordings were obtained at 1, 5, 6 and 7 months after birth. Measurements occurred under general short-term anesthesia for immobilization with isoflurane 2%. As previously reported,[25] the ABR was elicited by bone-conducted clicks of 100 microseconds with alternating polarity and a repetition rate of 11.7 clicks per second (Synergy version 14.1; Viasys Healthcare, Surrey, UK). The frequency characteristic of the bone conductor (RadioEar B-71, New Eagle, USA) showed a maximum output at 2 to 4 kHz. The bone conductor was fixed on the forehead in the midline with an elastic strap to ensure proper contact. A subcutaneous needle electrode was placed on one of both mastoids and a reference and a ground electrode on the nasal bridge in the midline. Ipsilateral ABR responses were passed through a 100- to 3000-Hz filter. Impedance of the electrodes was kept below 6 kΩ. One thousand responses to the clicks were averaged for each run.

The recording of the ABR in sheep resulted in a series of 5 identifiable waves. Waves III and IV were often seen as a complex, and remained the most consistently observed response at near-threshold level (Figure 1).Threshold was determined by lowering the intensity in 10 dB down until the III to IV peak complex disappeared. The stimulus intensity was then increased in 5 dB steps until a clear peak complex could be identified repeatedly. This minimal stimulus intensity for which the ABR waveform was evident by replication was considered as the threshold response value (dB peak equivalent sound pressure level). Measurements of ABR variables (absolute peak latencies and interpeak intervals) were assessed at a click intensity of 40 dB above the ABR threshold level of each subject. This intensity was used in order to compensate for individual differences in hearing thresholds and for the influence of sensation level on interpeak interval.[26, 27] Comparison of replicate recordings at threshold and at click intensity of 40 dB above threshold confirmed test-retest reliability in all cases (with a maximum of 0.2-millisecond difference between replications for peak III or V).[28] Single recordings were excluded from the analysis when interference of electromagnetic sources or the presence of excessive artifacts or noise obscured the ABR pattern, thereby rendering the reliability of correct identification of individual peaks doubtful. Two blinded observers (ALS, SMS) trained in ABR recording techniques completed all ABR measurements and identified the ABR parameters visually by consensus. Peaks of waveforms that appeared consistently within and across subjects were serially numbered (I-V) based on the recording of vertex upward deflections (Figure 1).[29, 30] The peak latency was graphically defined by the intersection of lines extended from the positive and negative slopes of the peaks.[28] Absolute peak latencies were referenced to stimulus onset. Interpeak intervals were determined from the respective peak latencies.

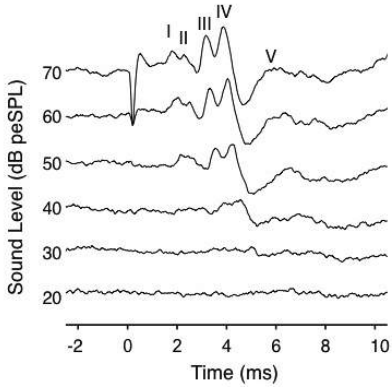

Figure 1. Auditory brainstem response waveform.
A representative sample of traces from a lamb of the sham group shows averaged traces at increasing levels of click presentation (dB peSPL), with the finding of an ABR threshold at 30 dB peSPL.ABR indicates auditory brainstem responses; peSPL, peak equivalent sound pressure level.

Statistical analysis

Independent sample *t* tests were applied to compare perilymphatic cytokine concentrations in the LPS and sham groups at 125 days GA, as well as ABR thresholds, peak latencies, and interpeak intervals at each of the 4 measurement moments (1, 5, 6, and 7 months). When it could not be clearly assumed that the outcome was normally distributed, the nonparametric Mann-Whitney *U* test was applied. Because of multiple comparisons and the likelihood of incorrectly rejecting a null hypothesis, a Bonferroni adjustment was made for peak and interpeak latencies relative to peak. In addition, ABR thresholds, latencies, and interpeak intervals were analyzed longitudinally relative to research groups over the age period from 1 to 7 months, whereby a mixed models procedure with random intercept was applied. SPSS 18.0 was used to analyze the data. Data are given as mean ± standard error of the mean (SEM), with a p-value less than .05 being considered statistically significant.

Results

Proinflammatory cytokines in perilymph

The nonparametric Mann-Whitney *U* test was applied for comparison of cytokine concentrations between groups because of not normally distributed data. Cytokine analysis of the perilymphatic fluid samples showed in the LPS group a significantly elevated concentration of IL-8 of 23.5 pg/ml compared to the sham group (P = .004; Table 1). No IL-6 could be found within the fluid samples of the LPS or sham groups. An elevated concentration of sICAM of 11.3 pg/ml was observed in the LPS group relative to the sham group (P = .005).

Table 1.

		IL-6		IL-8		sICAM	
Group	N	Mean	SEM	Mean	SEM	Mean	SEM
Sham	6	0.0	(0.0)	0.3	(0.3)	0.3	(0.2)
LPS	5	0.0	(0.0)	23.8‡	(6.8)	11.6‡	(4.3)

Abbreviations: IL, interleukin; LPS, lipopolysaccharide; sICAM, soluble intercellular adhesion molecule.
‡(Mann-Whitney, P <0.01).

Auditory brainstem response thresholds

The LPS-treated group had consistently statistically significant elevated threshold values by about 10 dB at 1 (df = 24, t = 2.276, P = 0.035), 5 (df = 27, t = 3.029, P = 0.005), 6 (df = 24, t = 3.682, P = 0.001), and 7 (df = 26, t = 4.919, P <0.001) months of age and over all time (df = 1, 32.1, F = 21.0, P <0.0005) compared to the sham group (Figure 2). Indicative for stable hearing thresholds, no significant changes in thresholds with increasing age were observed within groups.

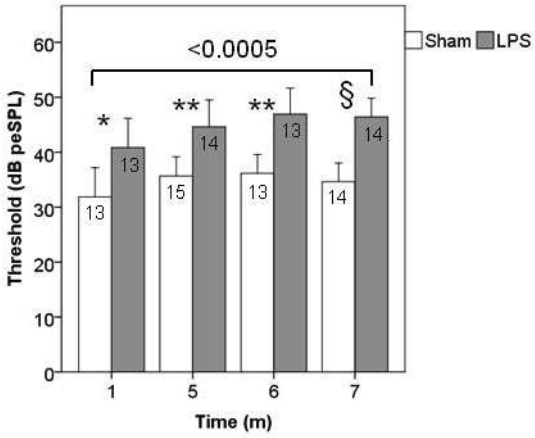

Figure 2. Mean ABR thresholds (dB peSPL) according to intervention and time of measurement at 1, 5, 6, and 7 months of age. Data are given as mean ± SEM. *P* values compare sham versus LPS at the individual time intervals (sham vs LPS, independent sample *t* test, * *P* < .05, ** *P* < .01, § *P* < .0005) and over all time (sham vs LPS, mixed models analysis).
ABR indicates auditory brainstem response; LPS, lipopolysaccharide; m, months; peSPL, peak-equivalent sound pressure level; SEM, standard error of the mean.

Auditory brainstem response latencies

Grouping all data obtained over different moments, absolute latencies in the LPS-treated group were significantly longer for peaks I (*df* = 1, 29.8, *F* = 6.2, *P* = .018), II (*df* = 1, 99, *F* = 6.0, *P* = .016) and V (*df* = 1, 31.4 , *F* = 19.6, *P* <0.0005) than those in the sham group (Figure 3). This effect was the most prominent at 1 month after birth, and only for the latency of peak V, we observed a consistent significant difference during all individual moments of observation between the LPS and sham groups. Longitudinally, in time, no changes in peak latencies were observed in either the LPS or the sham group, which would have been indicative of maturational aspects.

Interpeak intervals

Interpeak interval I to V was consistently longer for the LPS group relative to the sham group (*df* = 1, 24.8, *F* = 11.9, *P* = 0.002) throughout the total observation period, which can be explained by the longer III to V interval (*df* = 1, 32.5, *F* = 17.9, *P* = <0.0005) (Figure 4). Longitudinally, in time, a significant shortening of the interpeak latencies of interval I to III (*df* = 1, 71.0, *F* = 21.2, *P* <0.0005) and I to V (*df* = 1, 68.1, *F* = 18.0, *P* <0.0005) were observed in the sham group, indicative of normal maturational changes in ABRs.

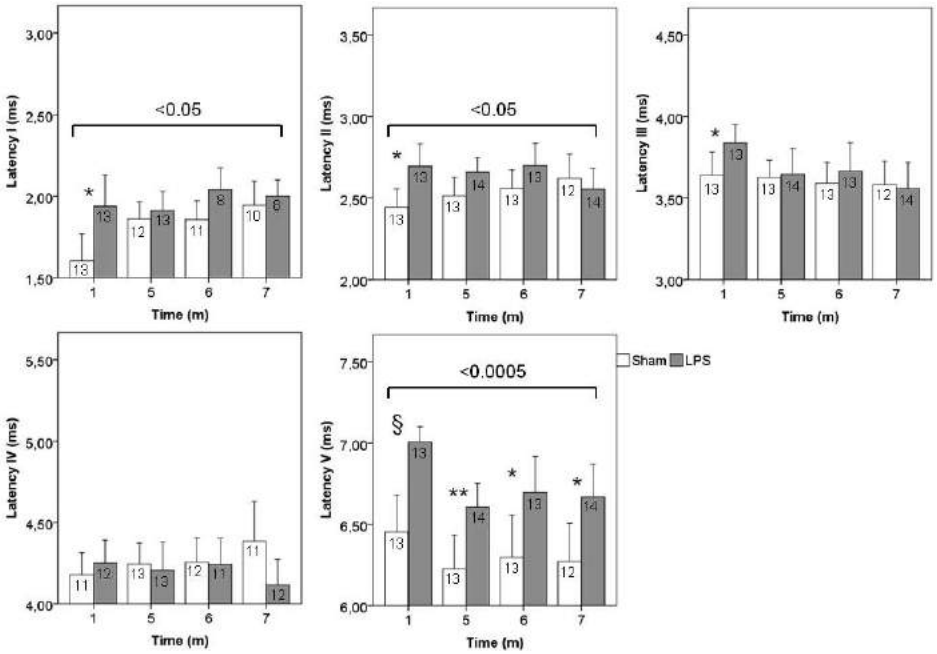

Figure 3. Mean peak latencies (in milliseconds) according to sham or LPS group at 1, 5, 6, and 7 months of age. Data are given as mean ± SEM. *P* values for comparison of the effect of intervention groups at the individual time intervals (sham vs LPS, independent sample *t* test, * *P* < .05, ** *P* < .01, § *P* < .0005) and over all time (sham vs LPS, mixed models analysis). After Bonferroni correction, the differences were statistically significant at 1 and 2 months for latencies V. LPS indicates lipopolysaccharide; SEM, standard error of the mean.

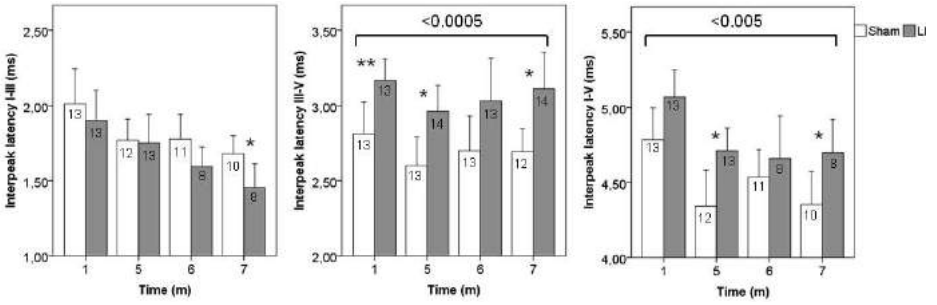

Figure 4. Mean interpeak latencies (in milliseconds) according to sham or LPS group at 1, 5, 6, and 7 months of age. Data are given as mean ± SEM . *P* values for comparison of the effect of treatment groups at the individual time intervals (sham vs LPS, independent sample *t* test, * *P* < .05, ** *P* < .01) and over all time (sham vs LPS, mixed models analysis). After Bonferroni correction, the difference was no longer statistically significant for interpeak latency I to V at 7 months. LPS indicates lipopolysaccharide; SEM, standard error of the mean.

Discussion

In this study, we tested the hypothesis that chorioamnionitis can affect the auditory pathway in terms of inflammatory changes of the fetal perilymph and altered hearing outcome after birth in a sheep model. A significant elevation of IL-8 and sICAM was demonstrated in fetal perilymphatic samples taken from the preterm lambs exposed to intrauterine LPS injection indicative of an inflammatory response. Interleukin 8 is known to be produced in response to a variety of stimuli such as LPS and provokes attraction and activation of neutrophils.[31] Cellular adhesion molecules facilitate the extravasation of leukocytes from the systemic circulation and have been demonstrated within the cochlea during inflammatory processes of the inner ear.[32, 33] No IL-6 was found, which could be explained by the timing of perilymph sampling at 14 days after intrauterine LPS injection. Previous experiments in this model showed in fetal cord blood a temporarily elevation in IL-6 within 5 hours after intrauterine LPS exposure, which was no longer significant after 24 hours.[22]

From our experiment, we cannot rule out that the elevated level of perilymphatic IL-8 and sICAM were originating from the cerebrospinal fluid (CSF) by diffusion due to cytokine related increased permeability of the blood-brain barrier.[34] The latter is supported by the results of cytokine analysis in CSF from preterm and term-born infants exposed to clinical or histological chorioamnionitis by Laborada and Nesin.[35] They observed an elevated level of IL-8 into the CSF of infants exposed to histological and clinically chorioamnionitis sampled within 24 hours after birth.

In the second experiment of our study, a small but statistically significant auditory threshold elevation of about 10 dB was measured in the group of LPS-induced chorioamnionitis when compared to sham treatment, in addition to a prolongation of peak V and interpeak interval I to V and III to V during the 7 months of observation after birth. Because of the resemblance of the ABR waveforms of sheep and other species,[29] the earlier ABRs are assumed to represent electrical activity in peripheral regions of the auditory pathway (eg, 8 nerve and cochlear nuclei), whereas the later waves will represent neuronal activity in central nuclear regions of the midbrain.[36] Considering this, the observed elevation of ABR threshold in combination with prolongation of interpeak intervals III to V and thereby I to V in the LPS group suggest an impairment of the central auditory brainstem function.[37, 38] Besides this, a significant elongation was found for peak latency I in the LPS group compared to sham treatment, which is supportive of a cochlear origin. Comparing this outcome with the described inflammatory response found into the perilymph, we cannot rule out that an LPS induced chorioamnionitis does have a deleterious effect on both the central nervous system as well as the cochlea.

So far, only Amin and Wang evaluated a similar hypothesis by analyzing electrophysiological hearing results in relation to chorioamnionitis.[39] In a cohort of premature born infants, they found no association between the existence of a histological chorioamnionitis and

alterations in ABR peak latencies within 48 hours after birth.[39] These contradictory findings between the latter study and our ABR measurements can be explained by differences in sample size, species, the inflammatory agent or dosage, or even timing of measurements.

Clinical evidence supporting our findings have been published by Suppiej et al. They analyzed neurodevelopmental outcome, including hearing loss, in preterm born children after a pregnancy complicated by histological-confirmed chorioamnionitis[12]. By parental questionnaires at 18 months of corrected age, hearing loss was scored as positive when the child had hearing difficulties requiring hearing aids. By this method, they found a higher relative risk of having hearing problems in the group of histological chorioamnionitis than in the control group (RR 2.76; 95 % confidence interval:1.64–4.64). These findings do support our results, although the observed ABR threshold elevation in our study of about 10 dB in the LPS group is small compared to hearing losses in children for whom amplification with hearing aids is recommended (> 35dB).[40]

To explain the detected auditory electrophysiological alterations after intrauterine LPS several mechanisms can be proposed. The induction of a chorioamnionitis at 111 days' GA coincides with a period of decreasing peak latencies during normal maturation. By the effect of the inflammatory products, central development could be halted. Several studies have shown altered gene expression of the fetal brain and subsequent abnormal neural development by elevated local cytokine levels after maternal LPS infection.[41–43] Another possible explanation is an interference with normal myelinization of the developing brain[44] or deleterious effects to central nervous system cells[18, 22, 45] by inflammatory mediators such as nitric oxide (NO) and peroxynitrite[46] resulting in changes in neural cell maturation and patterning.[47]

The presented study has several limitations. First, the ABR results in the sham and LPS-exposed animals were obtained by temporary immobilization by general anesthesia with 2 % isoflurane. Isoflurane is known to exert inhibitory and excitatory influence on neural transmitter systems.[48] Stronks et al. demonstrated in a guinea pig model the effect of isoflurane anesthesia on the central and peripheral auditory system.[49] They demonstrated a significant suppression of ABR amplitude, increased ABR threshold and latencies at concentrations of 2 %, and suppression of compound action potential amplitude and increased thresholds at higher isoflurane concentrations.[49] However, using this type of anesthesia in our study in both the LPS and control lambs, the influence of isoflurane on the ABR parameters would be the same in both the groups. Clinical data about ABR measurement with and without anesthesia would be needed to test its effect on sensory function, in particular, in preterm babies. Second, the magnitude of the described effect of LPS on ABR outcomes is modest. It is a plausible conclusion that the induced effects depend on LPS dosage. A positive correlation between the intrauterine LPS concentration and the magnitude of the systemic inflammatory response has already been demonstrated by Kramer et al.[22] A higher dosage of LPS could provoke a different effect. Third, it is possible that by

the application of a bone-conducted, click-evoked stimulus with a output maximum at 2 to 4 kHz, damage to lower and higher frequencies might be overlooked. Auditory brainstem response measurements with frequency-specific stimulation at 0.5 and 1 kHz might than be considered.

In conclusion, this study describes both the findings of fetal perilymph sampling and the postpartum repeated bone-conducted ABR measurements in a sheep model following LPS-induced intrauterine inflammation. Our hypothesis that a chorioamnionitis could result in a fetal inflammatory response of the perilymph and changes in ABR outcome after birth was confirmed. Whether these results can be generalized to the human situation experiencing a chorioamnionitis must still be determined.

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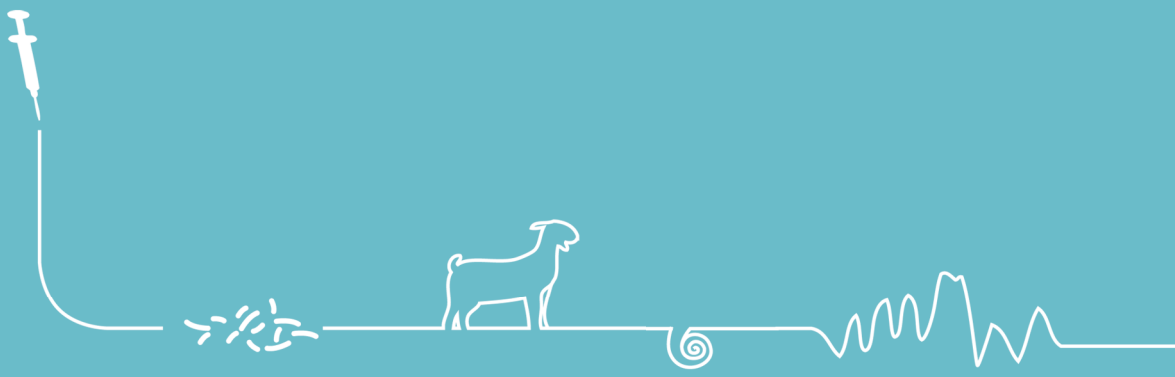

CHAPTER 4

Automated auditory brainstem response in preterm newborns with histological chorioamnionitis

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Abstract

Objective: We investigated whether histological chorioamnionitis is associated with adverse neonatal hearing outcome.

Methods: Two cohorts of very preterm newborns (*n* = 548, gestational age ≤ 32.0 weeks) were linked to placental histology and automated auditory brainstem response (AABR) outcome.

Results: In multivariable analyses, an abnormal AABR was not predicted by the presence of histological chorioamnionitis, either with or without fetal involvement (OR 1.4, 95 % CI 0.5–3.8, *p* = 0.54 and OR 1.1, 95 % CI 0.4–3.0, *p* = 0.79, respectively). Significant predictors of abnormal AABR included e.g. birth weight (per kg increase: OR 0.2, 95 % CI 0.0–0.6, *p* = 0.006), umbilical cord artery pH (per 0.1 increase: OR 0.7, 95 % CI 0.5–0.9, *p* = 0.005) and mechanical ventilation (OR 3.7, 95 % CI 1.2–11.6, *p* = 0.03).

Conclusions: Histological chorioamnionitis was not associated with adverse neonatal hearing outcome in two cohorts of very preterm newborns. Indicators of a complicated neonatal clinical course were the most important predictors of an abnormal hearing screening.

Introduction

The increased survival of very low birth weight newborns in recent years has drawn attention to their high risk of brain damage and the neuropsychological and behavioral difficulties at school age.[1, 2] Intra-amniotic inflammation/infection, as reflected by histological chorioamnionitis, is highly associated with preterm birth and its incidence increases with decreasing gestational age at birth.[3] In clinical studies histological chorioamnionitis has been associated with an increased risk for preterm white matter injury including periventricular leucomalacia (PVL) and cerebral palsy (CP).[4, 5] Moreover, several studies reported a relationship between exposure to chorioamnionitis and neurodevelopmental delay,[1, 2, 4, 6] although others have been unable to confirm this.[7, 8]

Antenatal inflammation has been shown to adversely affect the development of a range of fetal organs.[5] However, little is known regarding the association between antenatal intra-amniotic inflammation/infection and auditory impairment after birth.[9] A survey by Suppiej et al.[10] focused on an evaluation of the relationship between placental pathology and neurodevelopmental outcome and demonstrated an association between histological chorioamnionitis and the need for hearing aids and presence of speech delay among children born before 32 weeks gestational age at 18 months of age. A similar study in a population of infants born before 29 weeks of gestation failed to demonstrate such an association at two years of age.[11]

In the Netherlands, all newborns are enrolled in a national hearing screening program according to the recommendations of the Joint Committee on Infant Hearing.[12] The hearing screening for newborns admitted to the NICU includes a two-stage AABR measurement.[13] The target of the screening is to detect a potential unilateral or bilateral hearing loss of ≥ 35 dB.

We hypothesized that histological chorioamnionitis would adversely affect neonatal hearing outcome in preterm newborns. In this study, we evaluated whether histological chorioamnionitis with or without fetal involvement was a risk factor for abnormal AABR results in a high-risk population of very preterm newborns in two Dutch NICUs across two distinct time frames.

Material and methods

Study design and population

We studied the association between histological chorioamnionitis and hearing screening outcome in two separate cohorts. The first was a prospective cohort of inborn very preterm newborns (gestational age ≤ 32.0 weeks) admitted to the level III NICU of the Erasmus University MC–Sophia Children's Hospital in Rotterdam, the Netherlands be-

tween May 2001 and February 2003, described previously.[14–17] Trained research nurses unaware of results of placental histology prospectively collected relevant clinical data. The second cohort consisted of all very preterm newborns (gestational age ≤ 32.0 weeks) admitted to the NICU (level III) of the Máxima Medical Centre, Veldhoven, the Netherlands between 1 January 2009 and December 31 2010. The sub-cohort of singletons has been described previously.[16] Retrospective data retrieval was performed from maternal and neonatal medical charts and data were anonymised before storage. Newborns with severe congenital abnormalities were excluded from both cohorts.

Clinical characteristics

The following clinical parameters were recorded: maternal characteristics: maternal age, parity and gravidity; pregnancy characteristics: preterm premature rupture of membranes (PPROM), HELLP (intravascular hemolysis, elevated liver enzymes and low platelet count), pre-eclampsia (new onset hypertension (blood pressure > 140/90 mmHg or mean arterial pressure > 105 mm Hg) with proteinuria), and antenatal steroid administration (course of bethamethason 12 mg intramuscularly); delivery characteristics: gestational age (estimated by ultrasonography or otherwise by using the last menstrual period when reliable), delivery mode, sex, birth weight, Apgar score (at 1, 5 and 10 min) and umbilical artery pH and base excess. Neonatal outcome: presence of RDS (clinical presentation of expiratory grunting, sub- or intercostal or sternal retractions, nasal flaring, tachypnea, cyanosis in room air with or without apnea and characteristic radiographic appearance according to Giedion), need for any mechanical ventilation (SpO2 range 88–93 %), BPD (need for oxygen supplementation at a post-menstrual age of 36 weeks), hemodynamically significant PDA treated with drugs or closed surgically, NEC (Bell stage ≥2), clinical sepsis (clinical evidence of sepsis plus laboratory signs of infection, treated with antibiotics (early onset sepsis Rotterdam gentamicin with penicillin / Veldhoven gentamicin with augmentin, late onset sepsis (>72 u after birth) Rotterdam gentamicin with flucloxacillin / Veldhoven ceftazidime and teicoplanin), cystic PVL (cPVL), and severe intraventricular hemorrhage (IVH; grade 3 or 4).[14–17]

Placental histology

Placentas and membranes were fixed in formalin soon after delivery. Sampling was done according to a standard protocol and included at least two membrane rolls, two cross-sections of the cord, and three representative blocks of the placental disc. Tissues were embedded in paraffin until examination. Placentas were scored for presence of chorioamnionitis and additional fetal inflammatory response, according to international guidelines.[18]

AABR screening device

Hearing screening was performed by trained nurses on the ward, using an AABR neonatal hearing screening device (Rotterdam: ALGO Portable Newborn Hearing Screener, Natus Medical, Foster City, CA; Veldhoven: ALGO 3i Newborn Hearing Screener, Natus Medical, Foster City, CA). This device presented click stimuli at 35 dB hearing level (HL) monaurally at a rate of 37 pulses per second with a flat acoustic spectrum from 750 to 5000 Hz. Using an automated template-matching algorithm, electroencephalographic (EEG) activity for the presence or absence of an auditory brainstem response (ABR) was measured. When an individual trace matches the template with a statistically significant level of at least 99 %, a 'pass' result was obtained.[19] For each ear separately this resulted in a 'refer' or 'pass' testing, with a sensitivity of 97 % and specificity of 100 %.[20] AABR results were retrieved from the medical records and validated using the Dutch NICU Hearing Screening Database.[21]

Hearing screening program

The Dutch neonatal hearing screening program was a two-stage design and meets the standards of the Joint Committee on Infant Hearing Position Statement.[12] The first AABR was performed as close as possible to discharge from the NICU or referral to another NICU, medium or high care unit. In case of a 'refer' test result of one or both ears, a second test was performed at least two weeks later or when the infant reached term age. Infants failing the second AABR test were referred to an audiological center for further diagnostics of which data was not included in this study. Newborns who passed the first or second screening AABR-test were assumed to have normal hearing thresholds at that time. The final result was considered impaired when the newborn did not get a 'pass' on one or both ears after a second screening.

Ethical aspects

The study in Rotterdam was part of a research project on chorioamnionitis and neonatal outcome that was approved by the Medical Ethics Committee for Research on Human Subjects of the Erasmus University MC.[14] Written parental consent was obtained. For the Veldhoven cohort a waiver for ethical assessment and parental consent was provided by the local Medical Ethical Committee of the Máxima Medical Centre according to Dutch law, considering the retrospective and anonymized use of routinely collected medical chart data.[16]

Statistics

Continuous variables were expressed as mean and standard deviation, ordinal variables as median and interquartile range, and dichotomous variables as counts with respective percentages. Univariable between-group differences were accordingly tested using Student's *t*-test, Mann-Whitney *U*-test, and c^2-test, respectively. Variables associated with abnormal AABR screening in univariable analyses at $p<0.1$ were included in a multivariable logistic

regression model to predict abnormal AABR. A backward procedure was used for final model selection using a cut-off for variable inclusion of $p<0.1$. Placental pathology was included as a categorical variable (no HC, HC without fetal involvement, HC with fetal involvement). As it was the predictor under investigation, it was retained in the model irrespective of statistical significance, as was a hospital indicator to account for between-center differences. All analyses were performed using SPSS 20.0.0 (IBM SPSS Statistics, Chicago, IL).

Results

During the study periods, 301 newborns were included in Rotterdam and 357 in Veldhoven. In Rotterdam, 28 died before discharge and 25 did not have AABR data available, and were therefore excluded from further analysis. Nineteen newborns died in Veldhoven before discharge, and 37 did not have placental pathology available. Mortality between both centers was not statistically significant (Rotterdam 9.3% versus Veldhoven 5.3%, $p = 0.07$). AABR data was missing for one newborn in Veldhoven. Thus, 548 newborns were included in the analyses (Figure 1).

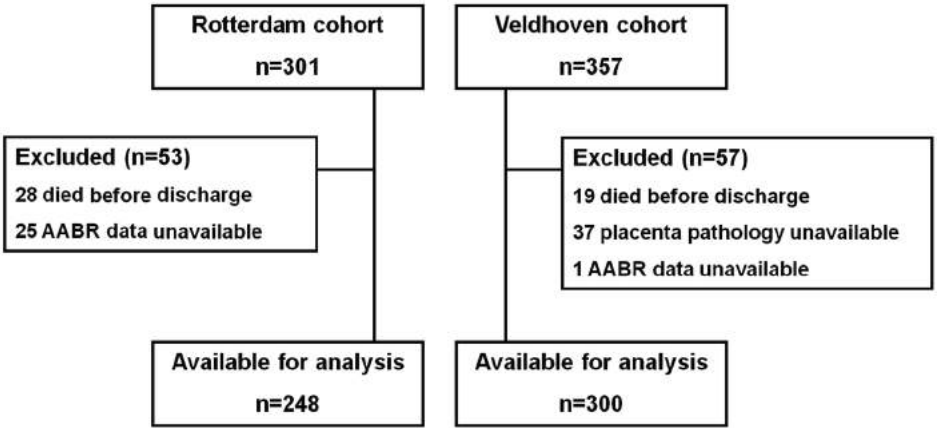

Figure 1. Flowchart of newborns included into the analysis of two cohorts.

Table 1 shows maternal and newborn characteristics according to placental pathology in both cohorts. Missing placental pathology in the Veldhoven cohort was significantly associated with a higher birth weight. Newborns whose placenta showed histological evidence of chorioamnionitis with or without fetal involvement were of lower gestational age, and were less likely to have been exposed to pre-eclampsia and HELLP syndrome, and more likely to be delivered vaginally and exposed to PPROM. In general, the associations between the maternal characteristics and histological chorioamnionitis were found in both centers.

Table 1. Maternal and delivery characteristics according to placental pathology.

		Rotterdam			Veldhoven			
Characteristics		No HC (n = 151)	HC (n = 46)	HCF (n = 51)	No HC (n = 165)	HC (n = 80)	HCF (n = 55)	Missing placenta (n = 56)
Maternal characteristics								
Maternal age (yrs)		30.8 (5.2)	30.8 (4.4)	30.7 (5.7)	30.5 (4.8)	30.8 (4.3)	29.7 (5.3)	29.4 (4.0)
Parity		1 (1–2)	2 (1–2)	1 (1–2)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Gravidity		2 (1–2)	2 (1–3)	2 (1–3)	1 (1–2)	1 (1–2)	2 (1–3)	1 (1–2)
Pregnancy characteristics								
PPROM		22 (15)	13 (28)	33 (65)***	36 (22)	28 (35)	38 (69)***	18 (32)
Pre-eclampsia		86 (57)	5 (11)***	2 (4)***	50 (30)	7 (9)***	0 (0)***	7 (13)
HELLP		53 (35)	3 (7)***	0 (0)***	30 (18)	3 (4)***	0 (0)***	4 (7)
Antenatal steroids		120 (81)	42 (91)	45 (88)	149 (90)	77 (96)	51 (96)	40/46 (87)
Delivery characteristics								
Gestational age (wks)		29.7 (1.6)	28.7 (1.8)**	28.4 (2.0)***	30.0 (1.5)	29.0 (2.0)***	28.6 (2.2)***	29.7 (1.8)
Caesarean section		118 (78)	18 (39)***	14 (28)***	98 (59)	22 (28)***	10 (18)***	21 (37)
Singleton pregnancy		115 (76)	27 (59)*	39 (77)	94 (57)	41 (51)	39 (71)	36 (65)
Male sex		76 (50)	27 (59)	25 (49)	80 (49)	51 (64)*	30 (55)	36 (63)
Birth weight (g)		1113 (307)	1242 (363)*	1201 (362)	1308 (347)	1294 (365)	1303 (364)	1406 (351)*
Umbilical artery	pH	7.23 (0.11)	7.25 (0.11)	7.29 (0.09)***	7.25 (0.12)	7.25 (0.13)	7.26 (0.10)	7.26 (0.14)
	BE (mmol/l)	-5.6 (4.8)	-5.7 (4.8)	-3.6 (2.9)*	-4.7 (4.9)	-4.8 (4.4)	-4.2 (3.9)	-4.4 (4.8)
Apgar score	1 min	6 (4–8)	6 (4–8)	6 (4–8)	6 (4–8)	6 (4–7)	6 (4–7)	7 (5–8)
	5 min	8 (7–9)	8 (7–9)	8 (7–9)	8 (7–9)	8 (7–9)	8 (7–9)	8 (8–9)
	10 min	9 (8–10)	9 (8–10)	9 (9–10)	9 (8–9)	9 (8–9)	9 (8–9)	9 (8–9)
Neonatal outcome								
RDS		84 (56)	25 (54)	29 (57)	68 (41)	27 (34)	16 (29)	25 (45)

Table 1. Maternal and delivery characteristics according to placental pathology. (continued)

	Rotterdam			Veldhoven			
Characteristics	No HC (n = 151)	HC (n = 46)	HCF (n = 51)	No HC (n = 165)	HC (n = 80)	HCF (n = 55)	Missing placenta (n = 56)
Mechanical ventilation	112 (74)	30 (65)	43 (84)	55 (33)	35 (44)	22 (40)	25 (45)
BPD	23 (15)	8 (17)	10 (20)	20 (12)	13 (17)	9 (16)	6 (11)
PDA	42 (28)	18 (39)	15 (29)	23 (14)	21 (26)*	15 (27)	13 (23)
NEC	1 (1)	3 (7)	4 (8)*	7 (4)	5 (6)	4 (7)	4 (7)
Sepsis	77 (51)	30 (65)	26 (51)	43 (26)	26 (33)	14 (26)	13 (23)
cPVL	5 (3)	2 (4)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Severe IVH	3 (2)	2 (4)	1 (2)	8 (5)	3 (4)	2 (4)	0 (0)
Gestational age at 1st AABR (wks)	33.7 (4.2)	32.6 (3.2)	32.8 (3.2)	32.1 (1.6)	32.1 (2.0)	31.6 (2.1)	32.2 (3.1)

Maternal and delivery characteristics of newborns according to placental pathology. Numbers represent number of newborns or mothers (+percentage) in which characteristic is present for dichotomous data, median and interquartile range for ordinal data, and mean ± standard deviation for continuous data. *p<.05, **p<.01, ***p<.001 vs. no HC or vs. included newborns (for newborns with missing placental sample). Abbreviations: AABR= automated auditory brainstem response; BE=base excess; BPD=bronchopulmonary dysplasia; cPVL= cystic periventricular leucomalacia; HC=histological chorioamnionitis; HCF=histological chorioamnionitis with fetal involvement; HELLP=hemolysis, elevated liver enzymes and low platelets; IVH= intraventricular hemorrhage; NEC=necrotizing enterocolitis ;PDA= patent ductus arteriosus; PPROM=preterm premature rupture of membranes; RDS=respiratory distress syndrome.

Table 2. Maternal and delivery characteristics according to AABR result.

		Rotterdam			Veldhoven	
Characteristics		AABR normal (n = 233)	AABR abnormal (n = 15)	AABR missing (n = 25)	AABR normal (n = 285)	AABR abnormal (n = 15)
Maternal characteristics						
Maternal age (yrs)		30.9 (5.1)	30.2 (5.7)	29.1 (4.8)	30.4 (4.8)	30.9 (5.1)
Parity		1 (1–2)	1 (1–2)	1 (1–2)	0 (0–1)	0 (0–1)
Gravidity		2 (1–3)	2 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)
Pregnancy characteristics						
PPROM		64 (28)	4 (27)	11 (44)	97 (34)	5 (33)
HC		43 (19)	3 (20)	5 (20)	126 (44)	7 (47)
HCF		47 (20)	4 (27)	5 (20)	52 (18)	3 (20)
Preeclampsia		90 (39)	3 (20)	7 (28)	56 (20)	1 (7)
HELLP		54 (23)	2 (13)	1 (4)*	33 (12)	0 (0)
Antenatal steroids		196 (85)	11 (73)	19 (76)	262 (83)	15 (100)
Delivery characteristics						
Gestational age (wks)		29.3 (1.8)	28.0 (2.2)*	29.9 (1.9)	29.5 (1.9)	28.2 (2.0)*
Caesarean section		141 (61)	9 (60)	13 (52)	125 (44)	5 (33)
Singleton pregnancy		170 (73)	11 (73)	16 (64)	166 (58)	8 (53)
Male sex		124 (53)	4 (27)	13 (52)	151 (53)	10 (67)
Birth weight (g)		1173 (328)	890 (307)**	1333 (444)*	1311 (356)	1155 (271)
Umbilical artery	pH	7.25 (0.11)	7.19 (0.10)	7.24 (0.10)	7.26 (0.12)	7.22 (0.10)
	BE (mmol/l)	-5.0 (4.6)	-7.7 (3.4)	-4.5 (4.2)	-4.6 (4.6)	-5.4 (4.0)
Apgar score	1 min	6 (4–8)	5 (4–8)	6 (4–8)	6 (4–7)	5 (2–6)*
	5 min	8 (7–9)	7 (6–9)	8 (7–9)	8 (7–9)	7 (6–8)*
	10 min	9 (8–10)	8 (8–9)	9 (8–10)	9 (8–9)	8 (8–9)
Neonatal outcome						
RDS		129 (55)	9 (60)	8 (32)	102 (36)	9 (60)
Mechanical ventilation		172 (74)	13 (87)	16 (64)	99 (35)	13 (87)***
BPD		36 (16)	5 (33)	4 (16)	39 (14)	3 (20)
PDA		65 (28)	10 (67)**	4 (16)	54 (19)	5 (33)
NEC		6 (3)	2 (13)	2 (8)	13 (5)	3 (20)**
Sepsis		124 (53)	9 (60)	9 (36)	74 (26)	9 (60)**
cPVL		8 (3)	1 (7)	0 (0)	0 (0)	0 (0)
Severe IVH		6 (3)	0 (0)	2 (8)	3 (1)	3 (20)***

Maternal and delivery characteristics of newborns according to AABR result. Numbers represent number of newborns or mothers (+percentage) in which characteristic is present for dichotomous data, median and interquartile range for ordinal data, and mean ± standard deviation for continuous data. *p<.05, **p<.01, ***p<.001 vs. AABR normal or vs. included newborns (for newborns with missing AABR). Abbreviations: AABR= automated auditory brainstem response; BE=base excess; BPD=bronchopulmonary dysplasia; cPVL= cystic periventricular leucomalacia; HC=histological chorioamnionitis; HCF=histological chorioamnionitis with fetal involvement; HELLP=hemolysis, elevated liver enzymes and low platelets; IVH= intraventricular hemorrhage; NEC=necrotizing enterocolitis ;PDA= patent ductus arteriosus; PPROM=preterm premature rupture of membranes; RDS=respiratory distress syndrome.

Table 2 demonstrates maternal and newborn characteristics according to AABR result. The incidence of abnormal AABR in Rotterdam and Veldhoven was 6.0 % and 5.0 %, respectively. Newborns with missing AABR data in the Rotterdam cohort less often had RDS and were born to younger mothers with less previous pregnancies and deliveries, whom less frequently had HELLP and more frequently had PPROM.

There were no significant differences in timing of AABR between groups based on placental pathology in both cohorts. In univariable analyses (Table 2), histological chorioamnionitis with or without fetal involvement was not associated with abnormal AABR. An abnormal AABR was generally associated with indicators of immaturity (lower birth weight and gestational age), perinatal stress (lower Apgar scores and umbilical cord artery pH), and illness severity (PDA, mechanical ventilation, NEC, sepsis, and severe IVH) in univariable analyses. Although the indicators of immaturity were not all statistically significant in both centers, all associations pointed in the same direction.

In a multivariable analysis, no association between histological signs of chorioamnionitis, either with or without fetal involvement, and abnormal AABR was found. Predictors of abnormal AABR included lower birth weight, lower umbilical cord artery pH, mechanical ventilation and NEC after multivariable adjustment. Omission of the hospital indicator from the model did not importantly alter the results (not shown) (Table 3).

Table 3. Final multivariable model for refer test result of AABR

Factor	OR (95 %CI)	P-value
Placental pathology (ref = no HC)		0.83
HC	1.1 (0.4–3.0)	0.79
HCF	1.4 (0.5–3.8)	0.54
Hospital (ref = Rotterdam)	1.6 (0.7–3.6)	0.27
Cord arterial pH (per 0.1 increase)	0.7 (0.5–0.9)	0.005
Birth weight (per kg increase)	0.2 (0.0–0.6)	0.006
Mechanical ventilation	3.7 (1.2–11.6)	0.03
Severe IVH	4.2 (0.8–22.0)	0.09
NEC	4.2 (1.1–15.9)	0.04

Multivariable analysis for newborns with a refer test result of the AABR. Abbreviations: CI=confidence interval; HC=histological chorioamnionitis; HCF=histological chorioamnionitis with fetal involvement; IVH=intraventricular hemorrhage; NEC=necrotizing enterocolitis; OR=odds ratio

Discussion

The present study demonstrated a significant relationship between indicators of a complicated neonatal course and abnormal AABR. Although significant associations between maternal characteristics and histological chorioamnionitis were observed, no association

was found between histological chorioamnionitis with or without fetal involvement and abnormal AABR in premature newborns.

Although preterm histological chorioamnionitis has been associated with a higher risk of white matter disease including PVL and CP,[4, 5] few studies have evaluated its relation with hearing outcome. In humans the third trimester and first postnatal months are a period of final structural and functional maturation of the cochlea and peripheral and central nervous system and thereby a vulnerable period to in utero and neonatal complications.[22] Suppiej et al.[10] demonstrated an association between histological chorioamnionitis and the need for hearing aids among children born before 32 weeks gestational age (relative risk 2.76; 95 % CI:1.64–4.64) and hypothesized that the inflammatory/infective processes could damage the cochlear maturation pathway. Whether this is related to the effect of neurotoxic inflammatory cytokines, the interference with myelination, or a negative effect on the developing brain of hypotension or intravascular coagulation is not clear.[10] Recently, the same research group evaluated hearing outcome by otoacustic emissions (OAE) and AABR in relation to histological chorioamnionitis in a population of 150 very low birth weight (VLBW) infants.[23] They found no association between histological chorioamnionitis and adverse hearing outcome at 6 months of age. Our findings are complementary to this observation, taking in account the differences in time of observation and the advantage of a larger sample size and data retrieval out of two distinct cohorts in our study.

An abnormal AABR was generally associated with indicators of immaturity (lower birth weight and gestational age), perinatal stress (lower Apgar scores and umbilical cord artery pH), and illness severity (PDA, mechanical ventilation, NEC, sepsis, and severe IVH) in univariable analyses in our study. After adjustment for confounders, only lower birth weight, lower umbilical cord artery pH, mechanical ventilation and NEC were significant predictors. An association between abnormal hearing screening and severe birth asphyxia as well as mechanical ventilation was also found in a similar study of Hille et al.[24] in a cohort of NICU infants born at <30 weeks of gestation and/or with a birth weight of <1000g. Whether the relationship with mechanical ventilation as confirmed by our study is related to respiratory insufficiency and hypoxia-induced cochlear [25, 26] or central auditory injury,[27] is a consequence of noise induced hearing loss,[28] or is due to coincidence with other risk factors, is not clear. The relationship between abnormal AABR and lower birth weight, as a correlate of lower gestational age, is hypothesized to be attributed to delayed maturation of the hearing system in premature infants.[29] In our study we also observed an association between the presence of NEC and abnormal AABR outcome. The incidence of NEC increases with decreasing birth weight and gestation.[30] Tobiansky et al. showed a significantly higher incidence of developmental morbidity in a cohort of very low birth weight newborns with NEC requiring surgery compared to matched controls.[31] As severe NEC is associated with hypotension, acidosis, inflammation and sepsis, but also with the

use of gentamicin antibiotics, all of these factors may contribute to the development of hearing impairment.[31]

We studied two large cohorts from two different centers, obtaining similar results. By temporal and geographical spacing between the two cohorts the generalizability of the results was increased. Limitations of the study are the retrospective nature of the data retrieval of the Veldhoven cohort with missing placental pathology outcomes as well as the missing values for AABR screening in the prospective Rotterdam cohort. Newborns with missing AABR data had lower birth weights and were born to mothers whom less frequently had HELLP, suggesting potential attrition bias. An explanation for this could be the discharge to another hospital of the newborn experiencing minor medical problems before reaching the age of their first AABR evaluation. Missing placental pathology in the Veldhoven cohort may have introduced additional bias as it was associated with higher birth weight for reasons unknown. For both cohorts, the degree of bias is expected to be small and is unlikely to explain the absent association between histological chorioamnionitis and abnormal AABR results. Several other factors have to be taken into account regarding the results of the AABR screening test performance. Because of the target range of the AABR, a normal test result will not rule out hearing loss at very low or high frequencies[12] or hearing impairment at <35 dB. However, hearing losses in these frequencies and of this magnitude will be of limited clinical importance. Also late onset hearing loss, which is described for children with a congenital CMV infection and survivors of Extra Corporeal Membrane Oxygenation (ECMO) will not be detected by hearing screening performed shortly after birth.

In conclusion, the presented study failed to demonstrate an association between histological chorioamnionitis with or without fetal involvement and abnormal AABR results. In a multivariable model lower birth weight, lower umbilical cord artery pH, mechanical ventilation and NEC were identified as significant predictors of abnormal AABR. Although chorioamnionitis is associated with certain adverse neurological outcomes, this study suggests that this does not translate into impaired neonatal hearing outcome.

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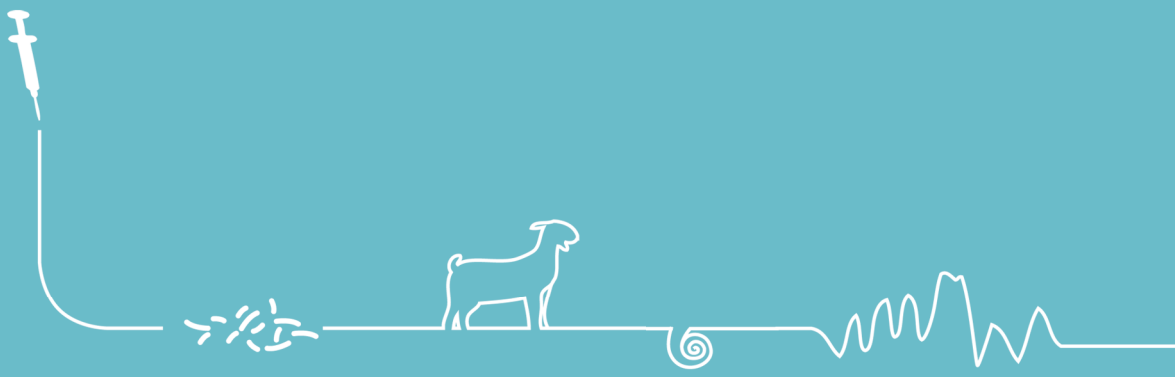

CHAPTER 5

Functional impairment of the auditory pathway after perinatal asphyxia and the short-term effect of perinatal propofol anesthesia in lambs

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Abstract

Background: Sensorineural hearing loss (SNHL) is a common feature in the postasphyxial syndrome in newborns. Several anesthetic drugs have been proposed to attenuate secondary neuronal injury elicited by hypoxia-ischemia. We hypothesized that propofol anesthesia reduces auditory impairment after perinatal asphyxia in comparison with isoflurane.

Methods: Twenty-three pregnant ewes were randomized to propofol or isoflurane anesthesia and sedation. The lambs underwent *in utero* umbilical cord occlusion (isoflurane $n = 5$; propofol $n = 7$) and were compared with sham-treated animals (isoflurane $n = 5$; propofol $n = 6$) at a gestational age of 133 d. For 8h after delivery by cesarean section, repeated auditory brainstem responses (ABRs) were recorded to obtain hearing thresholds, peak amplitudes, latencies and interpeak latencies.

Results: Significantly elevated mean thresholds, diminished amplitudes, and elevated latencies were observed in the asphyxia group relative to the control group through the observation period. Comparison of anesthetic treatment in the asphyxia group revealed a significantly lower elevation in threshold and less impairment in the ABR amplitudes and latencies during propofol anesthesia as compared with isoflurane anesthesia.

Conclusion: Our results support the hypothesis that anesthesia with propofol has a preventive effect on the functional changes to the auditory pathway in the event of perinatal asphyxia.

Introduction

Congenital sensorineural hearing loss (SNHL) is common. In the Western world, the incidence is 1–3 per 1,000 live births.[1–4] In the neonatal intensive care population, the incidence is even higher, with 2–4 per 100 live births.[3] Perinatal hypoxic-ischemic insults, which occur in 1–6 per 1,000 live full-term births, are one of the major risk factors for auditory impairment[5–7] and severe neurological sequelae in newborn infants.[8] Experimental studies suggest that there are already direct negative effects on hearing parameters within several hours to days after a peri- or postnatal event of severe hypoxia-ischemia.[9–11]

To date, little is known regarding the possibilities for intervention in this process. Several agents have been proposed to attenuate secondary neuronal injuries elicited by hypoxia, including anesthetic drugs.[12] Given the fact that each drug administered in a neonatal intensive care setting is associated with additional side effects for the newborn, the advantage of anesthetic drugs consists of their unavoidable need of administration in a situation after resuscitation and mechanical ventilation. This raises the question of whether it is possible to prevent or attenuate damage to the auditory pathway in asphyctic newborns by administering the right anesthetic drug.

Previous *in vivo* and *in vitro* studies demonstrated neuroprotective effects of several anesthetic drugs in hypoxic-ischemic insults.[13, 14] Of these, propofol is one of the most promising agents in mediating neuroprotection after hypoxic-ischemic insults in adults patients and animals.[15–18] However, in the developing brain, anesthetic agents have also been shown to induce apoptosis in newborn animal models.[19] So far, it is not known if propofol exerts a protective or aversive effect to the auditory pathway in perinatal asphyxia-induced functional hearing impairment. By comparing the effect of propofol, which exerts its effect via gamma aminobutyric acid and N-methyl-D-aspartate receptors,[20] with isoflurane anesthesia, which acts via sodium channel inhibition,[21] we tried to gain insight into protective strategies to diminish asphyxia-induced functional damage to the auditory pathway.

We hypothesized that (i) perinatal hypoxic-ischemia results in SNHL and (ii) perinatal propofol anesthesia reduces hypoxic-ischemia-induced SNHL as compared with isoflurane anesthesia. To test this hypothesis, late-preterm instrumented sheep fetuses were exposed to *in utero* umbilical cord occlusion (UCO) at 133 d of gestation (term: 150 d).[22–24] Fetal sheep of this gestational age are more susceptible to neurological damage after UCO than mid-gestation fetal sheep according to antenatal drug choice.[25] After delivery, fetuses were anesthetized with either propofol or isoflurane. Mothers received the same anesthetic drug during instrumentation and UCO of their fetus. The functional integrity of the auditory brainstem pathway was assessed by repeated auditory brainstem response (ABR) recordings of hearing threshold, peak amplitudes, latencies, and interpeak latencies during 8 h of observation.

Methods

Study design

This study was performed according to the guidelines of the Animal Care Committee of the University of Maastricht, which approved the protocol. Twenty-three time-mated Texel ewes and the preterm fetuses of both genders at a gestational age of 133 d were used. They were randomized according to an event of asphyxia by UCO *in utero* or sham procedure. Second, they were randomized for anesthetic/sedative treatment by propofol (sham: *n* = 6; asphyxia: *n* = 7) or isoflurane (sham: *n* = 5; asphyxia: *n* = 5).

Procedures

As previously reported,[26] pregnant ewes underwent cesarean section under general anesthesia by thiopental induction (15 mg/kg) and isoflurane anesthesia (1–2 %) or propofol both as induction (6 mg/kg) and general anesthesia (25 mg/kg/h). In both groups, anesthesia was supplemented by continuous remifentanyl infusion (3 µg/kg/min). After havening opened the uterus, the fetus was endotracheally intubated, and a vascular occluder was placed around the umbilical cord (OC16HD, 16 mm; In Vivo Metric, Healdsburg, CA). UCO was timed until the MABP dropped below 30 mmHg for 2min. This resulted in severe bradycardia (heart rate: < 30 bpm) or a complete cardiac arrest. Sham-treated animals underwent the same surgical procedures, except for the actual UCO. After delivery, the fetus was resuscitated according to current guidelines,[27] ventilated, and sedated either with propofol or isoflurane. After delivery, analgesia was obtained with intravenous remifentanyl (3 µg/kg/min), and sedation was maintained with propofol (1–3 mg/kg/h) or isoflurane (0.5–1 %) for 8h. The dosage was based on the clinical condition. The animals were killed at the end of the experiment with a lethal dose of intravenous T61 (Veterinaria AG, Zurich, Switzerland). Arterial and venous lines were used for continuous monitoring of MABP and heart rate and for repetitive hourly blood sampling to obtain pH values (Abbott i-STAT 1 Blood Gas Analyzer; Abbott Laboratories, Abbott Park, IL).

ABR recording protocol

During the 8h of observation, hourly recordings were conducted using an ABR device (Synergy version 14.1; Viasys Healthcare, Surrey, UK). The ABR waveform was elicited by a bone-conducted click-evoked stimulus of 0.1 ms per click, with a repetition rate of 11.7 clicks/s and alternating polarity. The conductor was fixed on the forehead with an elastic strap to ensure proper contact. A subcutaneous needle electrode was placed on the mastoid, and a reference and a ground electrode were placed on the nasal bridge in the midline. To elicit ABR wave morphology, responses were passed through a 100–3,000 Hz filter. Impedance of the electrodes was kept below 6 KΩ. A thousand individual responses to the clicks were averaged for each run (range: 1,000–1,008). Threshold was determined

by lowering the intensity in a 10 dB down, 5 dB up procedure until responses disappeared. The minimal intensity stimulus to which an ABR response was evident was considered as the threshold and expressed as dB peak-equivalent sound pressure level. Measurements of ABR variables (peak latencies, amplitudes and interpeak intervals) were assessed at a click intensity of 40 dB above the ABR threshold of each subject. In all cases, replicate comparisons confirmed test-retest reliability of all traces. Two independent blinded observers trained in ABR recording techniques completed the identification of all parameters visually. Peaks of waveforms that appeared consistently within and across subjects were serially numbered (I-V) based on the recording of vertex upward deflections (Figure 1).[28] The peak was graphically defined by the intersection of lines extended from the positive and negative slopes of the peaks. Because of a sloping aspect of peak V with an absent distinct negative through after the peak, the amplitude of peak V was calculated from the peak to the negative through following peak III. Absolute latency of a peak was referenced to stimulus onset. Interpeak latencies were determined from the respective peak latencies. If the peaks did not occur at the same latency on the replicate tracings, the ABR was considered to have low test-retest reliability, and the data were not used.

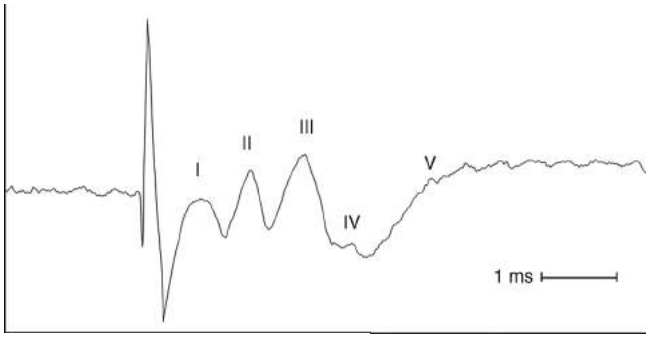

Figure 1. Bone-conducted stimulus ABR waveform pattern in the lamb model of 133 d of gestational age. The markers identify the waveform peaks (I-V) that were used for analysis. ABR, auditory brainstem response.

Statistics

Statistical calculations were made using SPSS 15.0 for Windows (SPSS, Chicago, IL). ABR parameter measurements were grouped according to time interval to facilitate comparison of the effects of interventions in time (time interval I: 0–3h, interval II: 3–5h, interval III: 5–8h after birth). Independent sample *t*-tests and repeated-measures ANOVA analyses over the three time intervals were applied to compare the effects of intervention and treatments. *P* values of <0.05 were considered statistically significant. Data are given as mean ± SEM.

Results

Physiological outcome

Observations were made in all 23 fetal sheep a mean gestational age of 133.8 ± 0.18 d. The groups did not differ according to gestational age, gender, or birth weight (3.5 ± 0.1 kg). The total UCO time needed until the mean fetal arterial blood pressure (MABP) dropped below 30 mmHg for 2min was not significantly different between the isoflurane group (11.3 ± 0.6 min) and the propofol group (10.7 ± 0.3 min). All animals developed bradycardia with a frequency of <30/min after UCO and needed resuscitation. The pH before birth was similar in all four groups (7.28 ± 0.02). The pH after birth decreased to 6.92 ± 0.05 in the propofol asphyxia group as compared with a pH of 7.15 ± 0.04 in the propofol control group. In the isoflurane group, the pH after birth was 6.88 ± 0.02 in the asphyxia group as compared with 7.11 ± 0.02 in the control group. In the asphyxia group, no significant differences were detected between anesthesia groups in MABP (isoflurane: 61.3 ± 6.2 mm Hg vs. propofol: 66.1 ± 7.3 mm Hg; $P = 0.300$) or heart rate (isoflurane: 187.4 ± 7.9 bpm vs. propofol: 181.6 ± 16.2 bpm; $P = 0.493$). There was a significant difference in the used dose of propofol ($P < 0.001$) and isoflurane ($P < 0.001$) between the control and asphyxia groups (Table 1).

Table 1: Dose of anesthetics used in lambs

	propofol/isoflurane dose
Propofol control	1.68 ± 0.08 mg/kg/h*
Propofol asphyxia	1.14 ± 0.06 mg/kg/h
Isoflurane control	0.86 ± 0.02 %*
Isoflurane asphyxia	0.57 ± 0.02 %

Data are given as mean ± SEM. P values to compare control vs. asphyxia per anesthetic treatment (control vs. asphyxia). *P < 0.001.

ABR recordings

ABR recordings were made in all lambs. Of the five positive ABR peaks, the first peak tended to coincide with the stimulus artifact at a click intensity of 40 dB above the ABR threshold (Figure 1). Wave III was the most prominent waveform peak in the ABR pattern, in contrast to a small peak IV, which was situated alongside the downhill slope of peak III.

Hearing threshold

The mean threshold was overall in time significantly higher in the asphyxia group than in the control group (control: 28.6 ± 1.4 dB peak-equivalent sound pressure level vs. asphyxia: 32.7 ± 1.1 dB peak-equivalent sound pressure level; $P = 0.034$). This difference was consistent by comparison of the individual time intervals. Differentiation in the asphyxia

group between anesthetics showed a significantly lower threshold in the propofol than in the isoflurane group (P = 0.013) (Table 2). The mean threshold was inversely correlated to both the pH (Pearson correlation coefficient: -0.34; P = 0.026) and the MABP (Pearson correlation coefficient: -0.30, P = 0.049).

Table 2: ABR parameters of waveform peaks according to interventions and treatment

		Control		Asphyxia		Control vs. Asphyxia
	Peak	Isoflurane(n = 5)	Propofol(n = 6)	Isoflurane(n = 5)	Propofol(n = 7)	P
Threshold		30.5(1.5)	26.9(2.2)	35.4(1.6)	29.8 †(1.1)	*
Amplitude	I	0.240 (0.027)	0.223(0.025)	0.192(0.026)	0.202(0.024)	NS
	II	0.279 (0.043)	0.281(0.012)	0.152 (0.030)	0.220 (0.018)	**
	III	0.840 (0.036)	0.864(0.059)	0.596(0.046)	0.720 †(0.045)	§
	V	0.045(0.008)	0.042(0.006)	0.028(0.005)	0.027(0.003)	*
Latencies	I	2.098(0.071)	2.068 (0.072)	2.136(0.054)	2.149(0.049)	NS
	II	3.232(0.095)	3.245 (0.057)	3.407(0.075)	3.380(0.061)	NS
	III	4.709 (0.103)	4.658 (0.099)	5.177(0.069)	4.930 † (0.065)	§
	IV	7.162 (0.117)	7.075 (0.119)	7.172 (0.067)	7.199 (0.070)	NS
	V	7.925 (0.108)	7.815 (0.123)	8.382 (0.056)	8.176 † (0.082)	§
Interpeak	I-III	2.749(0.058)	2.605(0.057)	2.794(0.049)	2.781(0.077)	NS
Latencies	III-V	3.009(0.103)	3.173(0.123)	3.295(0.060)	3.190(0.067)	NS
	I-V	5.757(0.110)	5.769(0.139)	6.078(0.049)	5.980(0.092)	*

Mean hearing threshold (dB peSPL), amplitudes (µV), latencies (ms) and interpeak latencies (ms) per peak (interval) according to sham control or asphyxia group and anesthetic treatment. Data are given as mean ± SEM. Comparison of isoflurane vs. propofol treatment within the control or asphyxia group († P < 0.05). P values compare control vs. asphyxia after correction for difference in anaesthetic treatment (control vs. asphyxia). ABR, auditory brainstem response; NS, nonsignificant; peSPL, peak-equivalent sound pressure level. * P < 0.05, ** P < 0.01, § P < 0.001.

Amplitudes

Peak amplitudes showed significantly lower values in the asphyxia group as compared with the control group for peaks II (control: 0.280 ± 0.022 µV vs. asphyxia: 0.182 ± 0.020 µV, P = 0.003), III (control 0.852 ± 0.034 µV vs. asphyxia 0.653 ± 0.034 µV, P <0.001) and V (control 0.144 ± 0.015 µV vs. asphyxia 0.127 ± 0.013 µV, P = 0.035) after correction for anesthetic treatment. The amplitude of peak IV was too small to allow reliable recording. In the asphyxia group, differentiation according to anesthetic treatment demonstrated significantly lower mean amplitude for peak III after isoflurane sedation in comparison to propofol (P = 0.038) during the total time of observation. Statistically significant differences between anesthetic treatments in the asphyxia or control group were not seen for the other peak amplitudes (Table 2).

Latencies

Significantly higher mean absolute latencies were seen after asphyxia as compared with the control group for peak III (control: 4.683 ± 0.070 ms vs. asphyxia: 5.064 ± 0.053 ms, P <0.001) and V (control: 7.870 ± 0.081 ms vs. asphyxia: 8.288 ± 0.052 ms, P <0.001). For peak II, the same tendency was observed, but this was not statistically significant (P = 0.052). These findings were consistent throughout the time intervals. Differentiation to anesthetic treatment in the asphyxia group showed significantly less elevated latency times in the propofol group than in the isoflurane group of peaks III (P = 0.015) and V (P = 0.046) (Table 2). In the control group, no significant differences were observed between anesthetic treatments.

Interpeak intervals

Mean interpeak latencies of intervals I-V were significantly higher in the asphyxia group than in the control group (control: 5.763 ± 0.087 ms vs. asphyxia: 6.033 ± 0.050 ms, P = 0.016) and were consistent during the whole time of observation. After differentiation to anesthetic treatment, no significant differences were seen between isoflurane and propofol sedation after correction for time interval in the asphyxia or control group (Table 2).

Discussion

SNHL is a common feature in the postasphyxial syndrome. In this study, we showed the effect of perinatal asphyxia on the auditory brainstem parameters and the impact of intervention by ante- and postnatal propofol vs. isoflurane anesthesia in a late-preterm lamb model.

Analysis of the ABR waveforms during the first 8 h after severe perinatal asphyxia demonstrated a direct impairment of the ABR parameters, which was consistent throughout the time of observation. In this period, a statistically significant elevated threshold was observed after asphyxia as compared with the sham-treated control group. However, the magnitude of the observed differences in threshold is small and around the precision of the testing procedure, with 5 dB steps. These effects could be more obvious in a more severe event of umbilical cord compression, which was shown to be feasible in the lamb model.[25] Nevertheless, the described effect of threshold impairment was supported by impaired peak amplitudes and latencies of earlier and later components of the auditory pathway. The presented results correlate with existing literature describing the effects of asphyxia to hearing function. Gunn *et al.* demonstrated prolongation of latencies and decreased amplitudes within hours to days after severe hypoxemia with acidosis in a fetal lamb model.[29] Jiang *et al.* confirmed this outcome in human term neonates.[9, 10] These

functional data together are in line with the findings of brainstem lesions with neuronal necrosis at sites concerning the auditory pathway after an event of perinatal asphyxia.[30]

To gain insight in protective strategies to diminish perinatal asphyxia-induced functional damage to the auditory pathway, we compared propofol and isoflurane anesthesia. In our experiment, we found a significantly smaller impairment of the ABR threshold after propofol as compared with isoflurane anesthesia. This protective effect of propofol was also present for the observed alterations of the amplitude of peak III and latencies of peak III and V. Although the described effects are small, these findings were reproducible throughout time of observation.

The neuroprotective effects of propofol after hypoxic-ischemic insults in adult animals have been proposed to be attributable to several mechanisms, including free-radical scavenging,[31] potentiation of gamma aminobutyric acid-mediated inhibition of synaptic transmission, and inhibition of N-methyl-D-aspartate-type glutamate receptor currents.[32–35] Which mechanism is responsible for the observed effects in our model of auditory effects in late-preterm lambs cannot be elucidated here.

A limitation of the current study is the short time of observation of the lambs after the insult, resulting from need for intensive care with ventilation and sedation. By this, a potential spontaneous (partial) recovery of auditory function in days to weeks after the hypoxic-ischemic insult, as described by Jiang *et al*.,[9, 10] could not be studied. Second, by administering the anesthetic drugs before birth to the maternal-fetal unit during cesarean section and postnatally to the newborns, we tried to imitate the situation during emergency cesarian section. However, with this approach, we cannot differentiate between the effect of propofol during or directly after the asphyctic event.

Our results support the hypothesis of a protective effect to the auditory function in lambs by antenatal anesthesia with propofol to prevent the negative effects of perinatal asphyxia. However, in the developing brain of newborn animals, propofol has also been shown to potentiate apoptotic neurodegenerative effects.[36–38] Whether this will outweigh the positive auditory outcome by propofol anesthesia must be considered when designing neuroprotective strategies to prevent auditory impairment after perinatal asphyxia.

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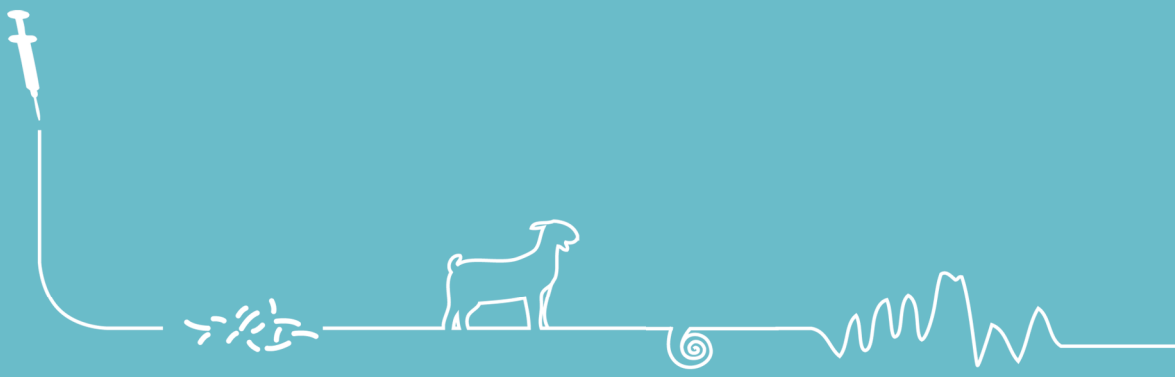

CHAPTER 6

7T MR imaging of the lamb temporal bone ex vivo

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Abstract

Purpose: Improving the resolution of temporal bone imaging is of major interest to demonstrate minor inner ear abnormalities and allow for the evaluation of the effect of interventional therapies in experimental protocols. In this study we assessed the feasibility of 7T MR temporal bone imaging in evaluating the inner ear structures of preterm lambs ex vivo.

Methods: 7T MR T2 spin-echo imaging was performed on three preterm lamb brains and skull bases (gestational age 120 days) postmortem. Because of immersion fixation of the skull the middle ear was filled with fluid. Acquisition time was 3.25 hours.The images of the six temporal bones were read by a neuroradiologist and anatomical inner ear structures were identified. Standardized measurements of fluid filled inner ear structures were performed by researchers independently to obtain dimensions. These values were tested for inter-rater reliability.

Results: Imaging quality was rated as good. All inner ear structures including cochlear scala, vestibulum, semicircular canals, niches, aquaducts and nerves could be identified. The smallest lumen diameters were found at the apex of the superior (0.37 mm, range 0.30–0.45 mm) and posterior semicircular canals (0.39 mm, range 0.26–0.55 mm). The correlation coefficient between raters was calculated to be high (r = 0.998).

Conclusion: High-resolution 7T MR imaging of the temporal bone is feasible and gives high quality detailed images of the fluid filled structures of the preterm lamb ex vivo. This tool may allow for the evaluation of subtle inner ear disorders by standardized measurement of individual structures and the effect of interventional therapies in experimental animal protocols.

Introduction

Radiological imaging of the labyrinth is an increasingly used diagnostic method in evaluation of both developmental and acquired disorders. Several factors can contribute to these disorders of the temporal bone such as infection[1], inborn anomalies [2], trauma, tumors, chemical teratogens, radiation exposure or drugs ototoxicity.[3] The occurrence of subtle or gross inner ear malformations leading to sensorineural hearing loss has resulted in studies directed towards improving high-resolution imaging techniques to assess the feasibility of cochlear implantation.[4] Magnetic fields of 4 to 9.4T have already shown to provide excellent anatomic imaging of the human brain by improving the signal-to-noise ratio (SNR) compared to lower magnetic fields.[5, 6] Visualization of the fluid filled inner ear by high increasing MRI field strengths is however challenging by the proximity of the bony skull base and air-filled areas leading to inhomogeneities in both the static (B0) and radiofrequency field (B1) fields.[7] By this limitation, attempts to visualize even more anatomic details by in vivo 7T imaging of the human membranous labyrinth (e.g. Reissner's membrane, scala media) have failed so far compared to 3T imaging, although 7T resulted in improved image quality compared to lower field strengths.[8, 9]

Imaging of the inner ear in experimental animals contributes substantially in assessing the effect of several interventions and treatments.[10, 11] Animal models with sheep have been used extensively in intrauterine and perinatal physiology and developmental research.[12, 13] The demonstrations of great anatomo-histological similarities between sheep and human auditory organs by morphometric and CT imaging studies,[14–16] makes this animal model of interest to study the effect of intrauterine and perinatal interventions by advanced (fetal) imaging techniques.[17]

The aim of this study was to evaluate ultra high resolution MR imaging as a diagnostic tool in the lamb model ex vivo. The application of standardized measurements of separate inner ear structures have been shown to be of additional value to reveal subtle inner ear malformations initially missed by visual inspection of temporal bone imaging.[18–20] By describing the fine inner ear structures by 7T MR imaging and applying standardized measurements of separate inner ear structures we evaluate the feasibility of this imaging technique in a ex vivo lamb model.

Material and methods

The study was approved by the Animal Ethics Committees at The University of Maastricht. Three time-mated ewes with singleton fetuses were used. Lambs were surgically delivered at 120 days gestational age (GA) (term = 150 days) and euthanized with 100mg/kg pentobarbital. The fetal head region was directly perfusion-fixated via the carotid arteries in

0.1M phosphate buffer (pH 7.4) and with 4 % paraformaldehyde (PFA) at 4°C for 24 hours and then stored in 30 % sucrose at 4°C before imaging. The Imaging was performed within this immersion fluid to overcome distortion by the existence of air into the middle ear.

7T MR imaging study

The scans where performed on a Bruker Biospin USR 70/30 (Bruker, Ettlingen, Germany). 3D-T2-weighted 7T MR images were made with whole brain and skull base coverage (quadrature head coil, diameter 72 mm, Bruker, Ettlingen, Germany) by usage of spin-echo sequence scan technique (slice thickness 0.2 mm, inter slice distance 0.2 mm, TE 78 ms, TR 2000ms, field of view 70x55x45 mm, matrix size 350x275x225, flip angle 114.2 °), with a total acquisition time of 3.25 hours. The maximal diameter of the skull was less than 70 mm.

Image processing and measurements

Post-processing of the image data into 3D multiplanar reconstructions (MPR) and image viewing were performed by using PACS Imaging Software (Carestream Health, Inc., Rochester, NY, USA). The MR scans of the three lambs depicting six temporal bones were rated in a random order by an experienced neuroradiologist to identify the following structures: presence of middle ear ossicles, scala tympani and vestibuli of the cochlea, vestibulum, lumen of the lateral, posterior and superior semicircular canals, oval and round niches, cochlear and vestibular aquaducts and presence of the seventh and eighth cranial nerve. The image quality was scored according to a 4 point scale (1 not visible; 2 visible, not diagnostic; 3 moderate but sufficient; 4 good quality).

Subsequent structural measurements of the inner ear structures were taken with electronic calipers under magnification by two trained researchers (Ear Nose and Throat residents) independently according to a standardized protocol in a double oblique view. The following structures of each ear were evaluated in a double oblique or parallel view. The length and width of the cochlear turns; length of modiolus; the diameter of the round and oval window; the length and width of the vestibule; the diameter of the lateral, posterior and superior semicircular canal at the apex, anterior and posterior crus; the width and length of the bony island of all semicircular canals; the diameter of the facial nerve, vestibulocochlear nerve, cochlear nerve with inferior vestibular nerve and the superior vestibular nerve were analyzed as shown in Figure 1.

Statistical analysis

Mean values, range and Pearson's correlation between raters of all measurements of the structures were computed using SPSS 21.0 software.

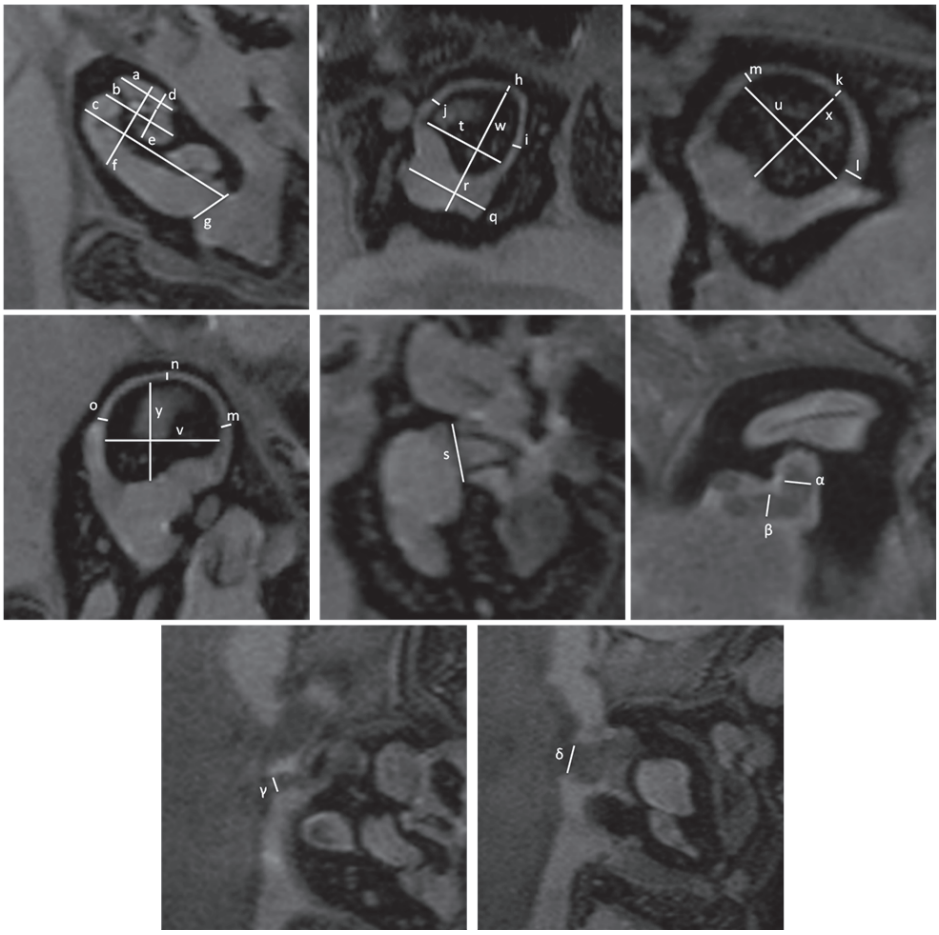

Figure 1. Multiplanar reconstruction of T2-weighted MR scans of detailed structures of the inner ear with markers of measurements. The length of the apical (a), middle (b) and basal (c) cochlear turn occurred in the slice with maximal length of these turns. In the same slice the width of the apical (d) and middle (e) turn and length of the modiolus (f) were measured perpendicularly, as well as the diameter of the round window (g). The measurements of the semicircular canals were made in slices along the long axis of the canal (double oblique). The diameter of the lateral semicircular canal was measured at the apex (h), at the centre of the anterior (i) and posterior (j) limb. In the same slice the vestibular width (q) and length (r) were derived. The diameter of the posterior semicircular canal was measured at the apex (k), just above the common crus at the medial limb (l) and at the same height at the lateral limb (m). The diameter of the superior semicircular canal was measured at the top (n), just above the common crus at the posterior limb (o) and at the same height at the anterior limb (p). The measurement of width (t,u,v) and length (w,x,y) of the bony islands were made perpendicularly in the same slides. The diameter of the oval window (s) was taken in a slide parallel to the slide of the cochlear turns. The diameter of the cochlear and inferior vestibular nerve (α) together and the superior vestibular nerve (β) were taken in a perpendicular view, at the outlet from the modiolus. The measurement of the facial nerve (γ) and vestibulocochlear nerve (δ) were made just after the root entry zone in a coronal view.

Results

All MR imaging studies were rated as having good imaging quality (mean score 4.0) in visualizing the inner ear structures. There were no signs of air in the fluid filled temporal bone. The round and oval window could be delineated in all cases by the presence of fluid inside the middle ear. The measurements of the fine inner ear structures are provided in the table (Table 1). In all lambs, a fluid filled lumen of the lateral, superior and posterior semicircular canals was present. The smallest lumen diameters were at the apex of the superior semicircular canal (0.37 mm, range 0.30–0.45 mm) and posterior semicircular canal (0.39 mm, range 0.26–0.55 mm). The cochlear turns were displayed very well by using a double oblique view (Figure 1) with separately depicted scala tympani and vestibule. Also the oval and round window, the cochlear and vestibular aquaduct as well as the facial and vestibulocochlear nerves were all identified in every ear of all lambs.

The measurements given by the two raters were compared (Figure 2). A Pearson's correlation coefficient (r) of 0.998 was obtained for all analysed parameters (paired sample t-statistic t = 0.305, df = 173, p = 0.761). The structures of the inner ear were sorted by category and scale to facilitate comparison between raters. Correlation between raters for the fine semicircular canal diameters were smaller (r = 0.612, t = 1.488, df = 53, p = 0.143) compared to larger structures as the bony island (r = 0.958, t = -0.671, df = 35, p = 0.507), cochlear turns, vestibulum and windows (r = 0.997, t = 0.633, df = 59, p = 0.529) and diameters of the cranial nerves (r = 0.995, t =-1.444, df 23, p = 0.162).

Discussion

In this study, we demonstrated the feasibility of ultra high resolution 7T MR temporal bone imaging ex vivo in a preterm lamb model. Our data showed high quality images of the small inner ear structures, in which all anatomical landmarks were identified and reference values of the anatomical structures of the lamb inner ear were obtained, despite its small calibers. A previous in vivo temporal bone study of intrauterine 1.5T MR imaging in fetal sheep was not able to identifiy the patency of the very small lumen of the semicircular canal at the same developmental age, despite its voxel size of 0.7 mm.[17] Our study by 7T MR had the advantage of a very high resolution of 0.2 mm and lack of movement artefacts by ex vivo scanning. This could be the reason why we were able to demonstrate the fluid filled lumen of all the semicircular canals of the inner ear. These findings could be of additional values in examining the effect of intrauterine or perinatal interventions in this animal model.

Normative measurements of the inner ear structures have been used to determine subtle inner ear malformations, initially missed by visual inspection of temporal bone

Table 1. Dimensions of inner ear structures of the lamb

Structure		Mean (mm)	Range Min-Max (mm)
SSCC			
Canal diameter			
	Anterior limb	0.47	0.36–0.57
	Posterior limb	0.43	0.36–0.51
	Top	0.37	0.30–0.45
Bony island			
	Width	4.99	4.27–5.80
	Length	4.95	4.61–5.45
LSCC			
Canal diameter			
	Anterior limb	0.45	0.33–0.58
	Posterior limb	0.42	0.30–0.55
	Top	0.44	0.36–0.61
Bony island			
	Width	4.03	3.56–4.40
	Length	4.92	4.47–5.25
PSCC			
Canal diameter			
	Medial limb	0.54	0.44–0.63
	Lateral limb	0.47	0.34–0.61
	Top	0.39	0.26–0.55
Bony island			
	Width	5.22	4.72–5.96
	Length	5.11	4.34–6.08
Cochlea			
Length			
	Basal turn	7.52	7.06–8.05
	Middle turn	3.76	3.43–4.02
	Apical turn	2.32	1.58–2.97
Width			
	Middle turn	1.46	1.21–1.87
	Apical turn	0.67	0.45–0.86
Modiolus	Length	4.04	3.76–4.58
Vestibulum			
	Length	4.79	4.50–5.54
	Width	1.33	0.57–1.91
Round window	Diameter	1.38	1.11–1.67
Oval window	Diameter	1.40	1.15–1.72
Facial nerve	Diameter	0.99	0.93–1.08
Vestibulocochlear nerve	Diameter	2.06	1.96–2.19
Cochlear/ inferior vestibular nerve	Diameter	1.14	1.05–1.19
Superior vestibular nerve	Diameter	1.03	0.95–1.10

Data are given as mean, minimum and maximum values. *SSCC* superior semicircular canal, *LSCC* lateral semicircular canal, *PSCC* posterior semicircular canal.

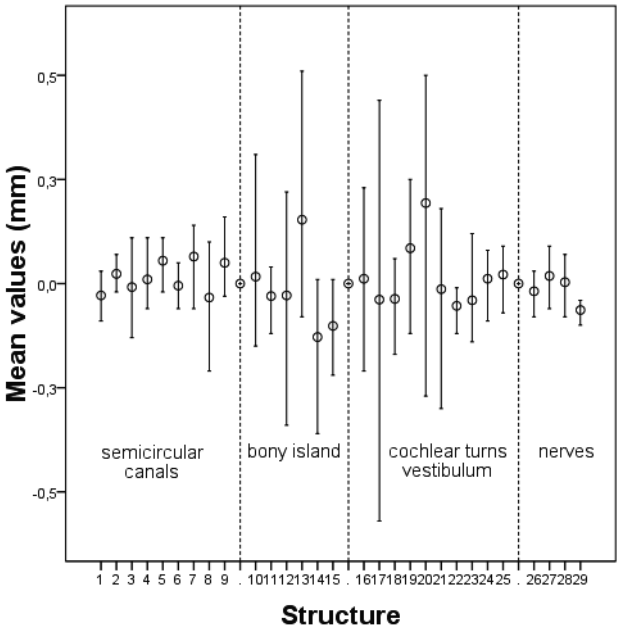

Figure 2. Mean difference (mm) between both raters of inner ear structures and range (diameter semicircular canals 1: SSCC anterior limb, 2: SSCC posterior limb, 3: SSCC top, 4: LSCC anterior limb, 5: LSCC posterior limb, 6: LSCC top, 7: PSCC medial limb, 8: PSCC lateral limb, 9: PSCC top / diameter bony island 10: LSCC horizontal, 11: LSCC vertical, 12: SSCC vertical, 13: SSCC horizontal, 14: PSCC vertical, 15: PSCC horizontal / diameter cochlear turns, vestibulum and window 16: apical turn width, 17: apical turn length, 18: middle turn width, 19: middle turn length, 20: basal turn length, 21: modiolus length, 22: oval window, 23: round window, 24: vestibulum width, 25: vestibulum length / diameter nerves 26: facial nerve, 27: vestibulocochlear nerve, 28: cochlear and inferior vestibular nerve, 29: superior vestibular nerve)

imaging[18–20]; a CT imaging study in children by Lan et al. identified subtle differences of the bony island width of the superior and lateral semicircular canal and in maximal height of cochlea in cases with congenital sensorineural hearing loss compared to a normal hearing population.[19] We presented normative measurements by 7T MR imaging of the inner ear structures with distances measuring twice the resolution size. The demonstration of lower correlations between raters for the very small distances of the semicircular canals indicates the approach to limitation of accuracy for evalution of these very fine structures.

One of the limitations of our study is the long acquisition time of more than three hours which was possible because of the postmortal research setting. Secondly, the specimen was immersed in fixation fluid during imaging. By this, visualization of structures will be influenced by the presence of liquid into the middle ear compared to air in living subjects.[7] Application in other conditions can be accomplished by optimizing imaging sequences and shortening imaging time, besides technical adjustments to overcome inhomogeneities in both the B0 and B1 fields.[21]

While the use of very high magnetic fields such as 7T is increasing in the research setting, clinical use is still in its infancy. The presented detailed data create opportunities for additional temporal bone imaging in the sheep animal model to study the effect of in utero complications and effect of interventions on the inner ear by 7T MR imaging.

Conclusion

In this study, 7T MR temporal bone imaging was feasible in evaluating the inner ear structures of preterm lambs ex vivo at high detail. We described normal diameters of the inner ear structures of a preterm lamb at 120 days GA, suitable as a future reference. This technique is a good candidate to evaluate the effects of intrauterine and perinatal interventions in labyrinthine disease and can provide additional detailed structural information in this model.

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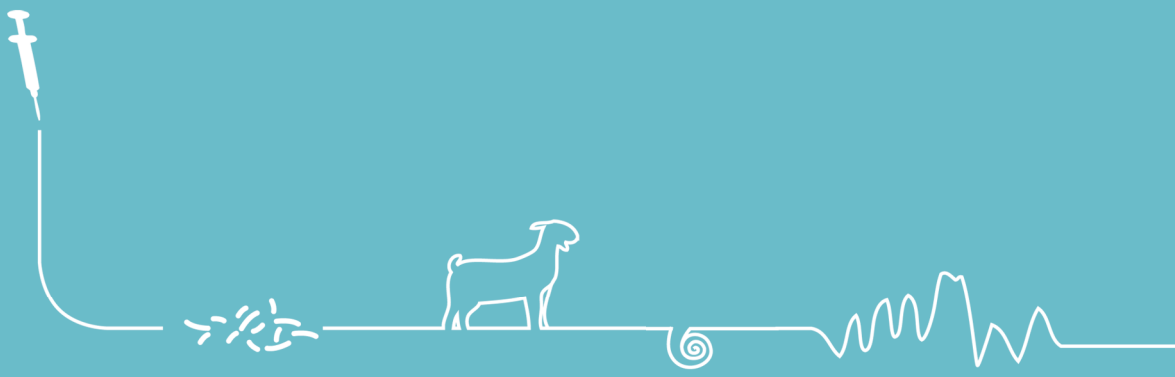

CHAPTER 7

General discussion and future directions

Discussion

According to recent estimates of the World Health Organization, more than 5% of the world's total population suffer from some form of disabling hearing loss (WHO, 2012). Besides this, sensorineural hearing loss is the most common congenital disorder, with a prevalence of 1 in every 1000 newborns in the Western World.[1] Congenital hearing loss does not only affect speech and language development in children, but also has economic consequences to the affected individuals and their families.[2]

The etiology of congenital hearing loss can be divided in genetic and nongenetic causes. Studies in hearing loss genes have increased rapidly with the advent of next-generation sequencing. However, it is in the group of nongenetic etiologies that large progress have been made in decreasing the chance for early hearing loss by the development of vaccines and improved antibiotic treatments.[3] In addition, hearing loss related to these nongenetic etiologies may be preventable and deserves particular interest to look for ameliorations in perinatal care. In this discussion we will focus at two entities; chorioamnionitis and asphyxia.

Chorioamnionitis

Chorioamnionitis induced adverse effects to the auditory system ?

Histological chorioamnionitis has been found to be associated with an increased risk for white matter disease in infants in clinical studies.[4–7] In premature born lambs, an LPS induced chorioamnionitis resulted in deleterious effects to the cerebral white matter, cerebellum, spinal cord and central nervous system cells.[8–10] We used this animal model to study the effects to the auditory system. By this, a fetal inflammatory response into the perilymph and small changes of ABR hearing parameters were demonstrated after birth which was explained by the deleterious effect of intrauterine LPS on both the central nervous system as well as the cochlea (chapter 4).

Only a few clinical studies addressed the effect of a chorioamnionitis to the auditory system before. Suppiej et al. showed a higher relative risk of having childhood hearing problems in preterm born children after a pregnancy complicated by histological chorioamnionitis.[11] In two clinical cohorts of very preterm newborns we found no association between histological chorioamnionitis and adverse neonatal hearing screening (chapter 3). However, our study outcome will not rule out hearing loss at very low or high frequencies, hearing impairment at <35 dB or late onset hearing loss.

Is a chorioamnionitis cytokine related multi-hit model applicable to hearing outcome?

A chorioamnionitis can induce an immune response in the fetus characterized by an elevation of pro-inflammatory mediators in the plasma and systemic activation of the fetal innate immune system.[12] Systemic cytokines and bacterial products might cross an intact

blood brain barrier.[8] Besides this, proinflammatory cytokines have been shown to increase the permeability of the blood-brain barrier by acting on the endothelial cells and tight junctions [13] which allows the entry of macrophages into the brain.[10] The inflammatory effects on the brain may contribute to an increased susceptibility to further insults eg asphyxia[14] since hypoxemia is a consequence of systemic fetal and maternal LPS administration.[15] This enables a hypothesis of a cytokine related multi-hit model of damage to the vulnerable perinatal central nervous system.

Is this multi-hit mechanism also applicable to the auditory system? The group of Hirose et al. exposed mice to ototoxic agents with or without prior LPS exposure. They demonstrated that LPS injection into the peritoneum of experimental mice compromised the blood-labyrinth barrier and induced a brisk cochlear inflammatory response with recruitment of mononuclear phagocytes into the spiral ligament, even in the absence of ototoxic agents.[16] While LPS alone did not affect hearing, animals that received LPS prior to ototoxic agents had worse hearing loss and more hair cell injury compared to those that received saline pretreatment.[17] They concluded that systemic LPS affects the blood-labyrinth barrier and causes a local inflammatory response by migration of leukocytes into the inner ear which is in line with our findings of a fetal perilymphatic inflammatory response after LPS induced chorioamnionitis (chapter 4). Where LPS pretreatment did not cause hearing difficulties in the experiments of Hirose et al., the combination with ototoxic agents did. This draws attention to the possibility of an inflammation mediated multi-hit model of the auditory system, in which a systemic (intrauterine) inflammation potentiates the effect of concomitant perinatal factors to affect the hearing capabilities of the newborn. This multi-hit hypothesis is of interest for future studies examining hearing outcome in relation to perinatal care.

Asphyxia

Perinatal hypoxia-ischemia induced adverse effects to the auditory system ?

Perinatal hypoxic–ischemic insults, which occur in 1–6 per 1000 live full-term births, is considered as one of the major risk factors for auditory impairment[18–20] and severe neurological sequelae in newborn infants.[21] The injury to the central nervous system is related to the degree, timing and duration of the hypoxic-ischemic event. [22 23] The inner ear seems to be less susceptible to hypoxia-ischemia compared to the central nervous system based on measurement of auditory brainstem responses (ABR).[18] Postmortem studies analyzing inner ear and auditory pathway histology in infants died after perinatal asphyxia showed pathological changes within the cochlea with loss of outer hair cells, and edema in the stria vascularis, degeneration of spiral ganglion cells, [24] besides alterations to the brainstem and auditory cortex.[25] However, because of the limited histological data in human infants with less severe HIE, the correlation between the degree of the hypoxic-ischemic event and histological changes to several parts of the auditory pathway is not clear. Besides this,

clinical studies do not agree on the exact role of perinatal asphyxia in hearing outcome. In chapter 3, we demonstrated in 2 cohorts of preterm born babies that the variables lower umbilical cord artery pH, besides lower birth weight, mechanical ventilation and necrotizing enterocolitis (NEC), were the most important predictors of an abnormal hearing screening outcome. However, generalizability of study results on this matter is hindered by differences in definition of variables like perinatal asphyxia, the studied cohort and confounding perinatal factors.

Perinatal intervention to minimize hypoxia-ischemia induced adverse outcome ?

After a hypoxic-ischemic insult, neuronal cell death is the consequence of immediate primary energy failure related to cellular hypoxia[26] and a secondary 'delayed phase' of neuronal cell death after a latent period of at least six hours.[27] Intervention during this critical period might stop this process.

For infants with hypoxic ischemic encephalopathy (HIE) the value of therapeutic hypothermia has been studied in pre-clinical models and several randomized clinical trials. From these studies, consistent evidence shows that hypothermia reduces the extent of neurological damage and improves survival without disability for term and late preterm newborns with moderate or severe HIE.[28] The neuroprotective effect of hypothermia have been proposed to be mediated by modification of programmed cell apoptosis, reducing cerebral metabolic rate, attenuating the release of excitatory amino acids, ameliorating the ischemia-impaired uptake of glutamate and lowering production of toxic nitric oxide and free radicals.[28] Mild hypothermia appears to be well tolerated in human newborns, without serious adverse effects.[29, 30] As a result, therapeutic hypothermia is now adopted as a valuable therapy to be offered to moderately and severely asphyxiated infants in several countries.

Is hypothermia feasible to prevent from ischemia induced damage to the auditory pathway ?

After a severe hypoxic event, progressive hearing loss as well as recovery of auditory function have been demonstrated suggesting a labile period after the primary insult, which might be of interest in designing rescue treatment. As mild hypothermia seems to be efficient in alleviating ischemic damage to the brain, one could argue if this effect is similar to the inner ear and auditory pathway. Several animal studies assessed ischemia induced inner ear damage and tried to emphasize the effect of hypothermia. Takeda et al. studied in a gerbil model ABR outcome and histological damage to the cochlear inner hair cells after transient cochlear ischemia.[31] Post-ischemic mild hypothermia started within 3 h after reperfusion was effective in attenuating hearing loss and inner hair cell damage. If this effect was exerted by a reduction of the effect of glutamate, which is thought to play in important role in ischemia-induced cochlear damage, or by cochlear protection through hypothermia induced attenuation of oxidative stress (iNOS/NO pathway), is not clear.[31]

In human neonates, the effect of therapeutic hypothermia in hearing outcome have been reviewed as secondary outcome in the meta-analysis by Jacobs et al, studying mortality and long-term neurodevelopmental disabilities in encephalopathic asphyxiated newborn infants.[28] In this meta-analysis, 7 studies were analyzed which reported on hearing disabilities in newborns (>35 weeks' gestation) with evidence of peripartum asphyxia and HIE which were treated by postpartum hypothermia compared to standard care (no cooling) (n = 720; hypothermia n = 396; control n = 324). Pooling of the short term results showed no significant effect of hypothermia on hearing loss at 1–2 years after birth (risk ratio 0.66 (95 % CI 0.35–1.26) irrespective of cooling technique. Only one study (n = 113) documented long-term hearing outcome at 6–7 years of age with no significant difference in hearing outcome between total body hypothermia and the control group (risk ratio 2.38 (95 % CI 0.26–22.20).[32] After publication of this meta-analysis also the TOBY study group reported on long-term hearing outcome of 181 children at 6–7 years of age and confirmed these findings.[33]

Some remarks have to be made about the outcome of this meta-analysis. The definition of scored hearing losses differed between the included studies. Secondly, between studies different subcategories of hearing losses were pooled to calculate overall relative risk of hearing difficulties; where for several studies the incidence of infants with hearing loss in need for hearing aids or worse were taken, for the Toby trial[34] the categories 'hearing losses not corrected by hearing aids' and 'no useful hearing' were taken without adding the number of infants with 'hearing loss requiring hearing aids'. However, it is unlikely that this would greatly influence the overall outcome.

Before concluding about the effect of hypothermia on hearing outcome in children suffering from HIE, one study by Mietzsch is worth to be mentioned. They evaluated shortly after birth otoacoustic emissions (OAEs) and ABR measurements in 9 HIE newborns which were randomized to hypothermia (n = 4) or standard care (n = 5) as a part of the NICHD study.[35] During the first week of life all newborns demonstrated that peripheral auditory function measured by DPOAEs was disrupted. The ABR waveform was delayed with normal interwave intervals suggesting cochlear insults. In two newborns treated by hypothermia, a faster recovery of DPOAE was seen throughout time of observation which would suggest that hypothermia may hasten recovery from a transient negative effect to the auditory pathway associated with HIE.[35]

From this data, we conclude that no beneficial effect to hearing outcome during childhood can be expected by the current studied therapeutic hypothermia protocols which have been demonstrated to improve neurological outcome in infants with moderate or severe HIE. However, we cannot rule out that hypothermia could hasten recovery of the auditory pathway from a primary hypoxic-ischaemic event during a labile period after the insult. This is of interest for future studies aimed to design preventive strategies.

Otoprotective agents in neonatal hypoxia-ischemia ?

Besides a protective effect of hypothermia to the brain in survivors of neonatal HIE, several drugs have been investigated for their potential beneficial effect. In chapter 6, we showed the effect of perinatal asphyxia to the auditory brainstem parameters in a late-preterm lamb model. A protective effect to the ABR parameters was demonstrated by ante- and postnatal propofol anesthesia compared to isoflurane anesthesia.

Experimental data is suggesting that hypothermia might extend the duration of the therapeutic window for HIE related neurological damage, and that drugs given during this time may be of additional effect in neuroprotection. Of these agents, several have been studied specifically in relation to hearing outcome in animal models of HI induced inner ear damage.

Magnesium Influencing glutamate mediated excitotoxicity, magnesium has been shown to be of additional value in overall better short-term neurological outcome in asphyxiated infants.[36] However, long-term benefits and safety has to be studied with and without concomitant hypothermia. A positive effect of magnesium to auditory function[37] and cochlear cell damage have been demonstrated in experimental animal studies of hypoxic-ischemia.[38]

Argon The noble gas argon as a non-competitive antagonism of the NDMA receptor, has been demonstrated to have neuroprotective properties in neuronal cell cultures[39] and in vivo models[40] of hypoxia-ischemia. Yarin et al. showed that exposure to argon hypoxia (5 % CO_2, 95 % Argon) protected the outer and inner hair cells in rats from hypoxia-induced damage compared to N_2 hypoxia.[41] Safety of argon ventilation in animals is the aim of current studies before conclusions about its application can be made.

Erythropoetin Of special interest is the application of erythropoietin (Epo). A recent randomized controlled trial in term infants with moderate to severe HIE showed an improved neurological outcome at 18 months of age after application of recombinant Epo (rhEPO) during the first weeks after birth.[42] Receptors of Epo have been demonstrated in the organ of Corti of guinea pigs[43] and rats.[44] In a neonatal rat models of hypoxia-ischemia the application of rhEPO resulted in reduced apoptotic changes within the ultrastructures of the inner ear and auditory pathway.[45] Several clinical trials using a combination of Epo with hypothermia are currently underway to provide additional information regarding the safety and efficacy of this potential therapy for HIE.

Perinatal otoprotective strategies

There are several considerations before drawing conclusions on the role of otoprotective strategies in perinatal medicine aimed at improving hearing outcome. First of all, there are often multiple perinatal factors simultaneously with separate but also interacting ef-

fects.[18] Second, the timing of exposure to these factors during gestation, and by this in auditory maturation, can greatly influence outcome since we know that preterm and term infants have a number of physiological differences and respond differently to perinatal complications.[46] Third, the degree and duration of the event will influence outcome. In case of hypoxia-ischemia, permanent damage to the auditory pathway will require prolonged hypoxia in combination with severe ischemia.[18] Considering intrauterine inflammation, the severity of a chorioamnionitis has to be taken in account in relation to hearing outcome; With high levels of LPS that mimic sepsis, damaging effects to the central nervous system have been demonstrated.[47, 48] By using a lower concentration of LPS, also beneficial effects have been observed in studies of brain injury.[49, 50] Fourth, susceptibility even within parts of the auditory system can vary; the vulnerability of the organ of Corti to hypoxic damage differs between the apical or basal part of the cochlea and between the inner and outer hair cells.[41] However, studies are not consistent on this outcome, possibly due to differences in experimental animal models.[51, 52] Therefore, potential otoprotective effects by cooling strategies and/or pharmacologic therapies have to be evaluated with respect to gestational age of the newborn, the degree of hypoxic-ischemia or infection, the timing of onset and duration of potential therapy. Moreover, when therapies will be designed for otoprotective reasons, the method of application of hypothermia or pharmacological agents can influence outcome. These facts point out the need for further research on this matter.

Considerations and future directions

In this thesis, we introduced an experimental animal model to study effects to the ovine auditory system. By this, the relationship between perinatal risk factors and hearing outcome was evaluated. However, the applicability of insights out of these experiments is not only restricted by the experimental conditions, but also by the lack of reference standards for this unique model. Besides this, generalization of outcome to daily clinical situation is limited by differences in the anatomy, physiology and lifespan between human and sheep. However, new opportunities are created to explore the effects of intrauterine and perinatal interventions in labyrinthine disease in near future study, as described by the application of ultra high imaging techniques of the temporal bone (Chapter 6). Besides this, the effect of otoprotective strategies in perinatal medicine is of great interest with regard to hearing outcome as described before. Recent advances in regenerative medicine suggest that stem cell transplantation may improve repair of the damaged brain.[53] Pre-liminary results in animal models have demonstrated beneficial effect to prevent from cochlear hair cell damage after transient ischemia of the gerbil cochlea.[54, 55] For this reason, more (pre)clinical studies are needed to clarify potential regenerative strategies to apply in newborns at risk for developing hearing loss.

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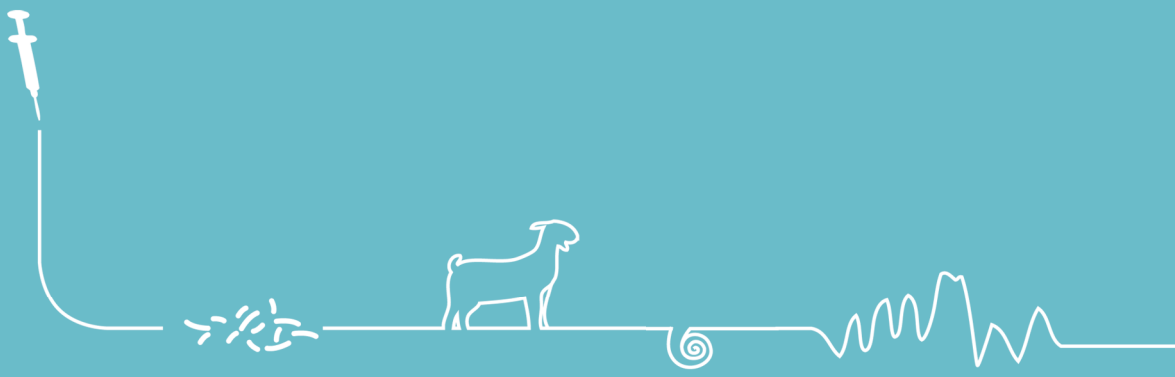

CHAPTER 8

Valorisation addendum

Valorisation addendum

Relevance and implications

Scientific relevance

According to recent estimates of the World Health Organization, more than 5% of the world's total population suffer from some form of disabling hearing loss (WHO, 2012). Besides this, sensorineural hearing loss is the most common congenital disorder, with a prevalence of 1 in every 1000 newborns in the Western World.[235] This thesis aims at the analysis of perinatal risk factors for hearing loss in childhood.

Since the introduction of national hearing screening programs in the western world, early identification of hearing loss has improved tremendously in the last decade.[275] Infants with a positive hearing screening tests are referred for total audiological examination. Depending on the etiology and level of hearing loss, the management of these children ranges widely; audiological, medical/surgical, educational and (re)habilitation methods, and child & family support.[276] Evidence is accumulating that early hearing loss interferes with or disrupts typical auditory cortical development by delaying or preventing the formation of neuronal connections and the maturation of cortical layers.[277] This underlines the need for early identification of hearing loss to be able to restore or improve auditory input to the brain within a sensitive period for auditory cortical development.[278]

Besides early identification of the actual hearing loss, a greater understanding of risk factors for congenital hearing loss is needed with regard to preventive strategies. Although the survival of preterm infants over the last decade has been improved by advances in perinatal care, the prevalence of major neonatal and long-term morbidities like hearing loss in this group has not significantly changed.[1] In this thesis we tried to gain insight in perinatal risk factors for auditory impairment.

First of all, the effect of an intrauterine LPS-infection to the auditory system was investigated (Chapter 2).In an animal model of LPS induced chorioamnionitis fetal perilymphatic inflammation was demonstrated as well as small changes to the auditory brainstem responses after birth (Chapter 3). The association between histological chorioamnionitis and adverse neonatal hearing screening was not confirmed in our study of two clinical cohorts of preterm born babies (Chapter 4). However, Hirose et al. recently showed that systemic LPS had direct effects on the blood-labyrinth barrier and induced signs of cochlear inflammation in a mice model.[242] While LPS alone did not affect hearing, animals that received LPS prior to ototoxic agents had worse hearing loss and more hair cell injury compared to those that received saline pretreatment.[243] Regarding the outcome of our studies, a multi-hit model was hypothesized to exert deleterious effect to the auditory pathway by intrauterine infection. This is of major interest for future studies examining hearing outcome in relation to intrauterine inflammation as we know that a chorioamnionitis is

present in ~10 % of term infants, in about 50 % of very low birth weights infants (VLBW) and up to 80 % of the extremely low birth weight infants (ELBW).[24]

Secondly, the effect of perinatal asphyxia on hearing outcome was analyzed in the second part of this thesis. Perinatal hypoxic–ischemic insults, which occur in 1–6 per 1000 live full-term births, is considered as one of the major risk factors for auditory impairment[17] and severe neurological sequelae in newborn infants.[14–16] However, clinical studies do not agree on the exact role of perinatal asphyxia and related variables in hearing outcome and evidence of intervention in this process to protect the auditory pathway from these effects is scarce. In a late-preterm lamb model, we showed the effect of perinatal asphyxia to the auditory brainstem parameters (Chapter 5). A protective effect to the ABR parameters was demonstrated by ante- and postnatal propofol anesthesia compared to isoflurane anesthesia. This outcome is in line with other experimental data suggesting that drugs given during a therapeutic window after a hypoxic-ischemic event may be of value in designing otoprotective strategies in near future. Keeping in mind that hearing impairment has major implications for quality of life, this underlines the medical and socio-economic relevance of this outcome.

In the last part of this thesis, the feasibility of ultra-high MR imaging was evaluated in the preterm ex vivo temporal bone. By the introduction of ultra high resolution MR imaging of the inner ear [52, 55] and the application of MR imaging even in fetal conditions[56, 57] new possibilities have been created to evaluate the inner ear in relation to several factors. We showed that 7TMR imaging gave high quality detailed images of the fluid filled structures of the preterm lamb temporal bone ex vivo (Chapter 6). This tool may allow for the evaluation of subtle inner ear disorders and the effect of interventional therapies in experimental animal protocols or in human clinical settings in future.

Economic relevance

Permanent hearing loss not only disrupts acquisition of spoken language, but also affects other domains of neurocognitive development, e.g. cognitive, social and academic functioning and by this constitutes a particularly serious obstacle to optimal development and education.[4, 5] It has been estimated that untreated deaf infants can cost society approximately $1,126,300 over the course of their lifetime.[279] The outcome of this thesis will contribute to a better understanding of the risk factors of hearing loss in children and by this, might help in potential otoprotective strategies in perinatal care. Reducing the incidence of neurodevelopmental morbidities by advances in perinatal care can be of great socio-economic value for the society.

Innovation

Within this thesis we explored scientific boundaries between research areas of congenital sensorineural hearing loss and perinatal medicine. By this, groundbreaking hypothesis

about the risk factors for hearing loss were analyzed and put in perspective. We introduced an original sheep model to analyze the effect of intrauterine inflammation and hypoxia-ischemia on the peripheral and central auditory pathway. The chronically instrumented fetal ovine model is widely used to observe fetal neurological outcome under a range of stimuli like LPS exposure[43–45] and hypoxia-ischemia.[46, 47] Application of this model to a broader set of questions concerning the perinatal and postnatal integrity of the auditory pathway is of interest to elucidate the relationship between perinatal factors and early hearing outcome.[50, 51]

Target groups

The unique topics within this thesis are of interest to a wide range of physicians. We have to keep in mind that in about 30 % of children with confirmed hearing loss at least one additional disability is demonstrated.[280] This underlines the multidisciplinary care for this group of children with neurodevelopmental disabilities including hearing loss. Also epidemiologists may be interested in this thesis. By nature, the highest risk for early hearing loss with a nongenetic etiology is amongst preterm NICU graduates. Most of the studies analyzing risk factors within this population do rely on retrospective data and frequently include only a small number of infants who develop sensorineural hearing loss. This is even more complicated by the dependency of several variables such as indicators of immaturity or illness severity. For the general population, a better understanding of risk factors for hearing difficulties in childhood is important in providing appropriate treatment and management for the deaf and hearing-impaired infants to help them to have similar opportunities in society alongside as other children.

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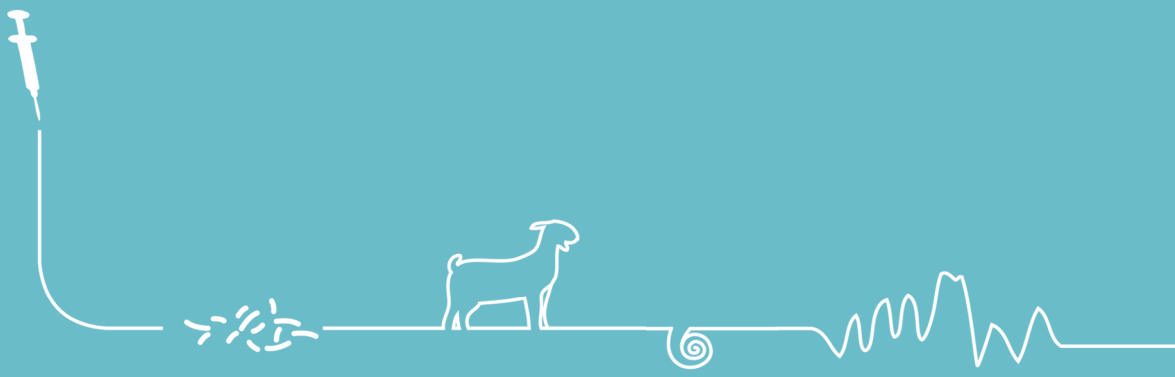

Summary

Summary

The studies included in this thesis address a number of topics concerning perinatal risk factors in relation to hearing outcome. Permanent hearing loss not only disrupts acquisition of spoken language, but also affects other domains of neurocognitive development, and by this constitutes a particularly serious obstacle to optimal development and education. So far, several indicators are generally considered as a risk factor for hearing loss in childhood and listed as such in the 'principles and guidelines for early hearing detection and intervention programs' by the Joint Committee on Infant Hearing.[1] However, other variables like chorioamnionitis and perinatal asphyxia are extensively studied because of their association with adverse perinatal outcome, but the significance of these factors in the onset of hearing loss is not clear and is the main focus of this thesis

In experimental animals, intrauterine inflammation can be produced by exposing the fetus to lipopolysaccharide (LPS), which is a product of the outer membrane of Gram-negative bacteria. In ***Chapter Two*** we report a review of the literature to elucidate the role of this toxin in congenital SNHL and to identify the pathogenesis and potential transmission routes. We speculate that different routes of exposure to LPS in utero can result in congenital inner ear damage.

To demonstrate the effects of an intrauterine infection by LPS to the auditory pathway, we introduce in ***Chapter Three*** a unique experimental model of chorioamnionitis in sheep. In this model, we demonstrate an inflammatory response in the fetal perilymph and deleterious effect to ABR parameters after birth by intrauterine LPS exposure compared to sham treatment. By this, we confirm the hypothesis of chorioamnionitis induced inflammatory effect to the fetal inner ear and functional impaired hearing outcomes after birth by an LPS induced-intrauterine infection.

We investigate the clinical relevance of a histological chorioamnionitis in neonatal hearing outcome in two Dutch cohorts of very preterm newborns in ***Chapter Four***. In multivariable analyses, an abnormal neonatal hearing screening by automated auditory brainstem response (AABR) was not predicted by the presence of histological chorioamnionitis, either with or without fetal involvement. Significant predictors of abnormal AABR included e.g. birth weight, umbilical cord artery pH and mechanical ventilation.

In ***Chapter Five*** we focus on the auditory features of the postasphyxial syndrome in newborns and the effect of anesthetic drugs to attenuate secondary neuronal injury elicited by hypoxia-ischemia. After delivery preceded by in utero umibilical cord occlusion, changes to serial auditory brainstem responses were observed in the asphyxia group relative to the control group. Comparison of anesthetic treatment in the asphyxia group revealed a significantly lower elevation in threshold, and less impairment in the ABR amplitudes and latencies during propofol anesthesia as compared to isoflurane anesthesia. These

results support the hypothesis that anesthesia with propofol has a preventive effect on the functional changes to the auditory pathway in the event of perinatal asphyxia.

In the study in ***Chapter Six*** we assess the feasibility of high-resolution MR imaging in evaluating the microstructures of the inner ear. We demonstrate in preterm ex vivo lambs high quality detailed images of the fluid filled structures of the labyrinth by 7T MR imaging. This tool may allow for the evaluation of subtle inner ear disorders and the effect of interventional therapies in experimental animal protocols in near future.

The studies included in this thesis were performed to improve our understanding of the perinatal risk factors for congenital hearing loss. Although the survival of preterm infants over the last decade has been improved by advances in perinatal care, the prevalence of major neonatal and long-term morbidities in this group has not significantly changed. A better insight into the factors related to adverse hearing outcome in childhood might help in designing potential otoprotective strategies in perinatal care. Keeping in mind that hearing impairment has major implications for quality of life, this can be of great socio-economic value for the society in near future.

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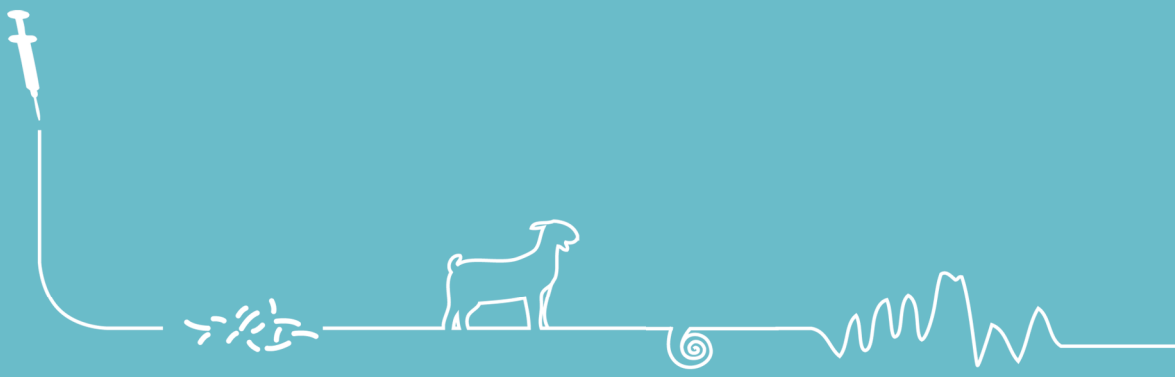

Samenvatting

Samenvatting

Dit proefschrift beschrijft aan aantal studies naar de perinatale risicofactoren voor gehoorverlies. Gehoorverlies in kinderen zorgt niet alleen voor een verstoring van de spraak- en taalontwikkeling, maar lijdt ook tot andere beperkingen in neurocognitieve ontwikkeling. Permanente gehoorschade vormt hiermee een belangrijke hindernis voor een optimale ontwikkeling en scholing. Door the Joint Committee on Infant Hearing zijn dusver meerdere risicofactoren voor gehoorverlies in kinderen aangewezen en opgenomen in 'principles and guidelines for early detection and intervention programs'.[1] Echter, voor diverse andere variabelen zoals chorioamnionitis en perinatale asfyxie, die eerder van negatief voorspellende waarde zijn gebleken voor de algemene perinatale uitkomst, is de exacte invloed op het gehoor niet eenduidig. Dit vormt dan ook het onderwerp van studie van dit proefschrift.

Lipopolysaccharide (LPS) is een molecuul van de buiten membraan van Gramnegatieve bacteriën. Door foetale blootstelling aan dit toxine kan een intra-uteriene ontstekingsreactie worden veroorzaakt in experimentele dierstudies. In ***Hoofdstuk twee*** wordt een overzicht gegeven van de literatuur die de relatie tussen intra-uterien LPS en het gehoor beschrijft. Om de potentiële rol van dit toxine in het ontstaan van aangeboren perceptief gehoorverlies te belichten worden routes van transmissie en de pathogenese toegelicht.

De effecten van een LPS gemedieerde intra-uteriene infectie op het auditieve system wordt verder onderzocht in ***Hoofdstuk drie***. Hiervoor introduceren wij een uniek chorioamnionitis diermodel gebruik makende van schapen. In dit model wordt aan de hand van analyse van de binnenoorvloeistof in foetale condities een ontstekingsreactie aangetoond ten gevolge van intra-uterien LPS blootstelling. Daarnaast worden functionele negatieve effecten gezien op het gehoor in de groep van LPS blootstelling ten opzichte van de controle groep door middel van BERA-onderzoek (Brainstem Evoked Response Audiometry / hersenstam-audiometrie). Hiermee kan worden bevestigd dat een LPS gemedieerde chorioamnionitis een foetale ontstekingsreactie kan geven van het labyrint en functionele effecten heeft op het gehoor na geboorte in dit model.

De klinische relevantie van deze bevindingen wordt geanalyseerd in ***Hoofdstuk vier.*** In een tweetal Nederlandse groepen van ernstig prematuur geboren kinderen wordt de relatie onderzocht tussen een histologisch bewezen chorioamnionitis en de uitkomsten van de neonatale screenende gehoortest. Hieruit blijkt een afwijkende gehoortest door middel van AABR techniek (Automated Auditory Brainstem Response) niet voorspeld te worden door de aanwezigheid van histologisch bewezen chorioamnionitis, al dan niet met foetale betrokkenheid. Significante voorspellers van een afwijkende gehoortest worden gevonden in o.a. het geboortegewicht, navelstreng pH en de noodzaak voor mechanische ventilatie.

In ***Hoofdstuk vijf*** wordt de nadruk gelegd op het gevolg van perinatale asfyxie op het gehoor en de interventie door middel van anesthesiologische farmaca om secundaire neuronale schade te verminderen ten gevolge van hypoxie-ischemie. In het pasgeboren schaap wordt na een periode van intra-uteriene navelstreng occlusie veranderingen waargenomen van hersenstampotentialen ten opzichte van de controle groep. In de asfyxie groep wordt tijdens propofol anesthesie een kleinere verslechtering gezien van de gehoordrempel en BERA piek amplitudes en latentietijden in vergelijking met isofluraan anesthesie. Deze resultaten ondersteunen de hypothese dat anesthesie met propofol een beschermend effect heeft op functionele gehoorveranderingen ten gevolge van perinatale asfyxie.

Hoofdstuk zes beschrijft de haalbaarheid van de toepassing van hoge resolutie MRI in het analyseren van de microstructuren van het binnenoor. Door middel van ex vivo 7T MRI beeldvorming van het os temporale van prematuur geboren lammeren worden de met vloeistof gevulde structuren van het labyrint in detail in beeld gebracht. De toepassing van deze techniek zou kunnen worden toegepast in de toekomst om subtiele veranderingen van het binnenoor te kunnen detecteren en kan van waarde zijn in de beoordeling van effecten van diverse effecten in experimentele diermodellen.

De in dit proefschrift beschreven studies zijn uitgevoerd om het begrip van perinatale risico factoren voor aangeboren gehoorverlies te vergroten. Hoewel door verbeteringen in de perinatale zorg de overleving van prematuur geboren kinderen is toegenomen gedurende de laatste tientallen jaren, is de prevalentie van morbiditeit op de korte en langer termijn niet evident veranderd in deze groep. Een beter begrip van factoren die kunnen lijden tot een negatieve gehooruitkomst in kinderen kan uiteindelijk bijdragen in de ontwikkeling van beschermende perinatale maatregelen gericht op gehooruitkomst .In ogenschouw nemende dat gehoorverlies grote gevolgen heeft op de kwaliteit van leven, kan dit inzicht van grote sociaal-economische waarde zijn voor de maatschappij in de toekomst.

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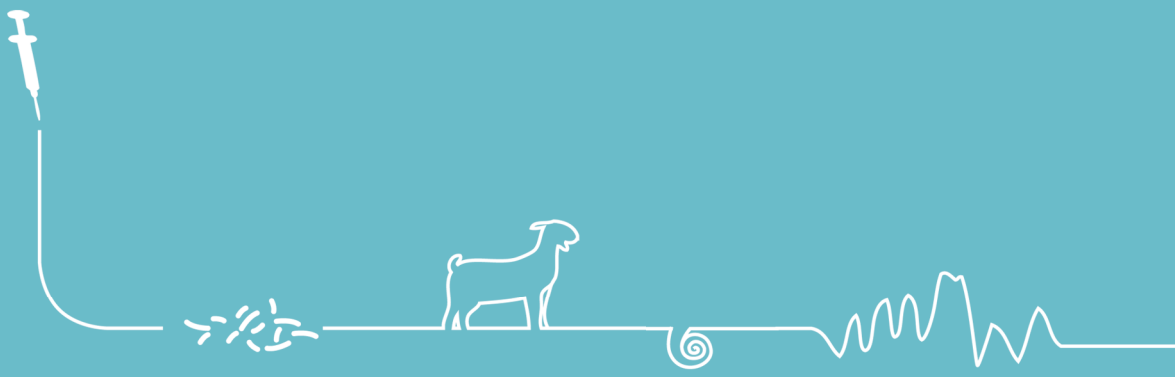

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List op publications

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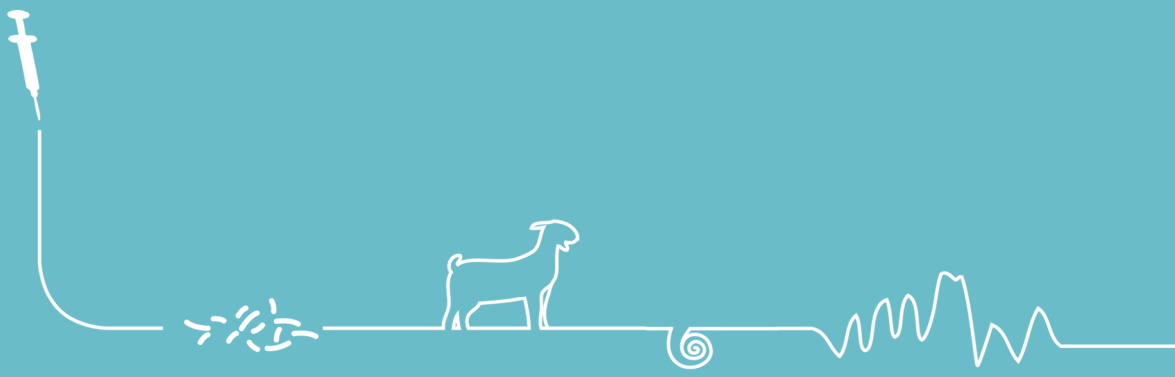

Dankwoord

Dankwoord

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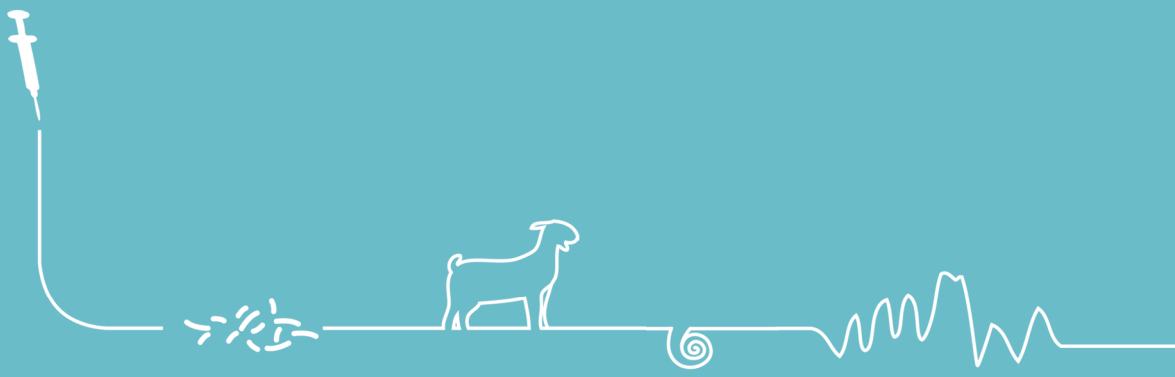

Curriculum Vitae

Curriculum Vitae

Adriana Leni (Diane) Smit werd op 21 november 1978 geboren te Delfzijl. Zij rondde in 1997 het VWO af aan het Fivelcollege te Delfzijl. Na het behalen van het toelatingsexamen startte zij haar studie Geneeskunde aan de Universiteit van Antwerpen. De 1e & 2e kandidatuur behaalde zij met onderscheiding en daarna zette ze haar studie voort aan de Universiteit Utrecht. Na het behalen van het artsexamen in 2005 werkte zij periodes als arts-assistent bij de afdeling Gynaecologie en Obstetrie van het Meander Medisch Centrum te Amersfoort, de afdeling Chirurgie van het Gelre ziekenhuis te Apeldoorn en in het Thoraxcentrum van het Erasmus MC te Rotterdam waar zij waardevolle chirurgische ervaring opdeed. Vanaf 2007 richtte zij zich geheel op de KNO en doorliep de opleiding Keel-, Neus- en Oorheelkunde in het Maastricht Universitair Medisch Centrum (MUMC+) onder prof. dr. B. Kremer en plaatsvervangend opleider prof. dr. R.J. Stokroos.

Haar interesse voor het proces en de toepassing van wetenschappelijk onderzoek werd aangewakkerd tijdens stages binnen de studie Geneeskunde op de afdeling KNO van de Massachusetts Eye and Ear Infirmary in Boston en tijdens haar onderzoeksstage op de afdeling Neonatologie van het WKZ te Utrecht. Binnen de opleiding KNO kreeg deze interesse vorm in het huidige promotieonderzoek. Na het afronden van haar opleiding in 2012 is zij gaan werken als staflid KNO in het Universitair Medisch Centrum te Utrecht. Ze richt zich met veel plezier op de otologie en pediatrische KNO, zowel in haar klinische werkzaamheden als op het gebied van de wetenschap. Ze woont in Utrecht, samen met haar vrouw Noëlle en hun kinderen Teun, Olivier en Josephine.