Quantification of histopathological changes in the inner ear of piglets with hypoxic-ischemic-encephalopathy following perinatal asphyxia

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Introduction

Hearing loss following perinatal asphyxia

Perinatal asphyxia results in hypoxic-ischemic encephalopathy (HIE) in 1-6/1000 of live human births, of which 10-60% of affected infants die. ~30% of the survivors suffer from mild to severe systemic and neurologic deficits, including sensorineural hearing loss (SNHL)[1].

Previous descriptive histological studies have suggested that perinatal asphyxia-induced SNHL is due to cellular damage in the peripheral auditory system[2-5]. However, several studies evaluating hearing thresholds of infants with HIE indicate that the peripheral auditory system remains intact. This is supported by the finding that the rat inner ear remains intact after birth asphyxia, suggesting that the observed hearing impairment is of central origin[6].

Currently, evidence of histopathological changes in the auditory system evaluated using quantitative methods following HIE-induced SNHL is limited, and the possible relationship to electrophysiological alterations in the auditory pathways is insufficiently investigated.

Research question: Does perinatal asphyxia impair auditory function in mammals, and does the dysfunction correlate with histopathological changes in the cochlea?

Research motivation

Children exposed to HIE currently undergo monitoring of hearing function until the age of seven in Denmark. Our results will contribute to the assessment of whether HIE patients are in risk of developing SNHL, ensuring that patients receive the most appropriate monitoring and treatment.

Methods

1. Piglet model of global neonatal hypoxic-ischemic encephalopathy

- Established model[7] of global hypoxia without surgical intervention, apart from intubation and umbilical vessel catheterization
- Individual differences in tolerance to hypoxia is taken into account by adjusting the oxygenation according to aEEG response and blood pressure

Figure 1. HIE induction in piglets

- Piglet physiology is comparable to human newborns and they develop encephalopathy similar to that seen in the asphyxiated term infant
- 32 Danish landrace pigs (~1.5 kg; age 18 hrs): 4 sham, 28 HI lesion
- Neurologic scoring both pre- and post-hypoxia

2. Electrophysiological assessment of hearing function

- Auditory brainstem response (ABR)

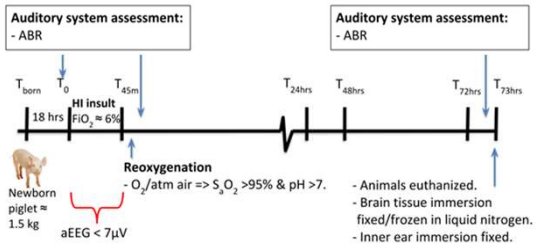
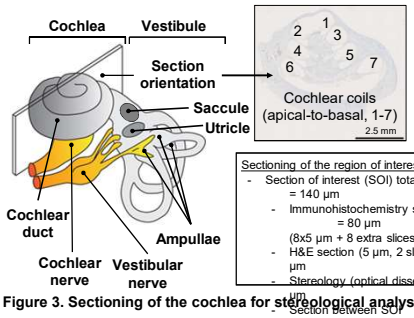


Figure 2. Workflow chart of the hypoxic-ischaemic insult and electrophysiological assessment of auditory function. At the beginning of the study, the 18-hr-old piglets were exposed to a HI insult lasting 45 min. During the insult, the F_iO_2 was kept at the lowest value at which the aEEG was below 7 µV but temporarily increased in cases of severe hypotension. Thereafter, the piglets were reoxygenated and monitored and kept alive for 3 days.

3. Histological analysis of the inner ear



- Apical cochlea: Low freq. hearing
- Basal cochlea: High freq. hearing
- Cells of the basal cochlea are generally more prone to damage

Figure 3. Sectioning of the cochlea for stereological analysis.

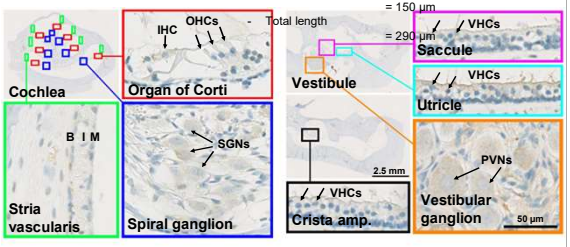
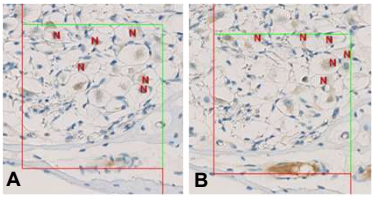


Figure 4. Potential cell types of the cochlea and the vestibular system damaged by HI lesion. IHCs, inner hair cells (auditory receptor cells); **OHCs**, outer hair cells (amplification); **M**, marginal cells; **I**, intermediate cells; **B**, basal cells (endocochlear potential generation) **SGN**, spiral ganglion neurons (relay sound information to the central nervous system); **VHCs**, vestibular hair cells (balance receptors); **PVNs**, primary vestibular neurons (relay balance information to the central nervous system).

Figure 5. Quantification of SGNs using the physical fractionator on scanned sections. Quantification of cell numbers is based on counting nuclei of SGNs that appear only in one of the two consecutive 5 µm paraffin sections (A,B; SGNs marked with *N;* scanned with Hamamatsu NanoZoomer) within the counting frames (VIS, Visiopharm).



Preliminary histological results

1. HI insult does not lead to extensive apoptotic cell loss in the inner ear

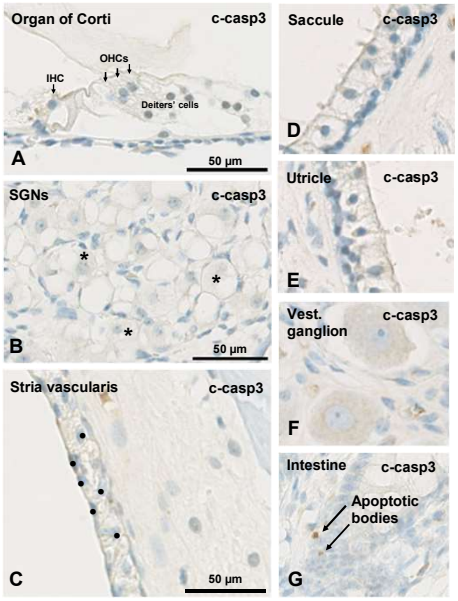


Figure 6. HI insult does not lead to apoptotic loss of cochlear cells. Representative paraffin sectioned piglet **(A)** organ of Corti, **(B)** SGNs, **(C),** Stria vascularis from the basal cochlear coil three days after the HI insult. Note that hair cells (*arrows* in A), SGNs (*asterisks* in B), and strial cells (*dots* in C) are maintained and lack the apoptotic marker c-casp3. **(D)** Saccule **(E)** Utricle **(F)** Vestibular ganglion **(G)** Positive control (intestine) of the c-casp3 immunostaining labels apoptotically degenerating cell profiles. *Abbreviations:* c-casp3, cleaved caspase 3; HI, hypoxic-ischemic; IHC, inner hair cell; OHC, outer hair cell; SGN, spiral ganglion neuron.

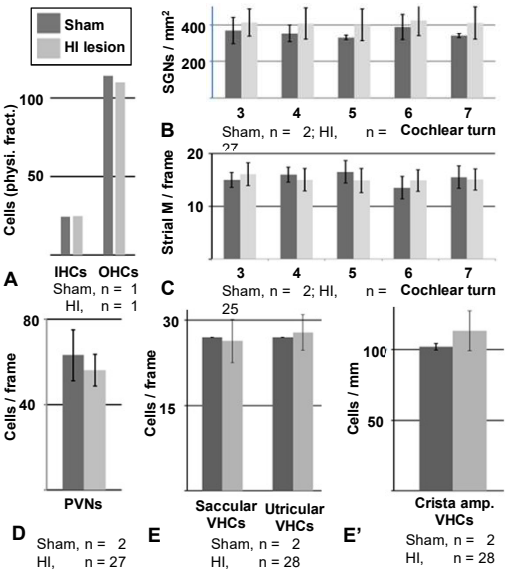


Figure 7. Preliminary quantification of cochlear cells does not reveal significant differences between the sham and the HI lesion. (A) Preliminary stereological quantification of hair cell number in sham (*dark gray*) and piglets subject to HI insult (*light gray*) show closely comparable results. Quantification of average number of **(B)** spiral ganglion neurons (SGNs), **(C)** strial marginal cells (M), **(D)** primary vestibular neurons (PVNs), and **(E,E')** vestibular hair cells (VHCs) with midcochlear sampling show no difference between sham (dark gray) and HI lesion (light gray) treatment groups. Counting areas: *SGNs* - bony capsule surrounding them; *PVNs* - 500x500 µm frame; *Saccular and utricular VHCs* - 250x50 µm frame along the epithelium; *Stria vascularis* - 100x200 µm frame along the epithelium; *Crista amp. VHCs* - length of the analyzed basement membrane. Error bars = S.D.

Preliminary conclusions

- No signs of cell loss observed in the piglet cochlea and vestibular organ following HI insult
  - Combinatory effects of other factors associated with birth asphyxia (aminoglycoside ototoxicity, incubator noise, low birth weight)?
  - Possibility of tissue processing artefacts in previous studies?
  - Sensorineural hearing loss following HIE may be of central origin
- Further research is required to ensure that HIE patients in risk of developing sensorineural hearing loss receive the optimal care, monitoring, and counselling

Acknowledgments

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