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GROWING UP WITH ONE EAR: CENTRAL AUDITORY STRUCTURE AND FUNCTION IN UNILATERAL EAR CANAL ATRESIA

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Growing up with one ear: Central auditory structure and function in unilateral ear canal atresia

Thesis for Doctoral Degree (Ph.D.)

By

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To my daughters Ellen and Magdalena, and to my husband Jonas.

Popular science summary of the thesis

Congenital unilateral, or single sided, ear canal atresia means that the external ear canal is missing on one ear since birth, leaving just blunt skin over bone instead of a canal leading to the eardrum and middle ear. The outer ear is often small and malformed. The condition prevents is sound from reaching the inner ear, which is normal in most of the cases, and results in profound hearing loss of a type that is conductive. It is also known that hearing loss in one ear, even if the other ear has normal hearing, leads to difficulties in awareness of sound source, and understanding of speech in a noisy environment. There are options to correct the hearing of an ear with atresia: bone conduction hearing devices connected to the skin of the skull, surgically implanted hearing aids or in some selected cases, reconstruction of the ear canal with surgery. However, it is not well known if there is a time window in childhood or adolescence to adapt to hearing aids, or any measure to advise for or against hearing aid treatment in individual cases.

The studies of the following thesis were designed to investigate if single sided ear canal atresia with associated conductive hearing loss on the affected ear has consequences on the development of the auditory system of the brain, if no hearing aid treatment is offered in childhood. Participants with ear canal atresia on one ear, and one normal hearing ear were included and examined to find out if the brain can functionally adapt to hearing with this ear only, and if there are any changes to the auditory system of the brain that can be visualized with an MRI scan of the brain.

In the first study, localization ability was studied in unilateral hearing loss, in participants with ear canal atresia. It is well known that two ears vastly facilitate localization ability, because the brain can analyze the difference in time and intensity of which the same sound reaches the two ears. In localization with only one hearing ear, the person is dependent on judging of the sound intensity whether it comes from the hearing side or not and the sound must then have the same intensity over time to be possible to predict. The other option includes the spectral shape cue, which is a feature of the outer ear to form sounds of shorter frequencies differently depending on their source location. In atresia, the hearing loss can be moderate or severe. In the localization task, it was shown that even if the hearing of the malformed ear is very reduced, even the severely weakened sound cues that reach both inner ears are helpful to localize sound source. The same study also tested the ability to understand speech in a setting with several voices talking at the same time. The participants with atresia had worse results than normal hearing test persons, but the level of hearing loss of the malformed ear in participants with atresia was not related to their level of performance in the speech understanding task.

In the second study, the participants with atresia went through MRI scanning of the brain. Here, we described a method to track the white matter pathway, the "acoustic radiation", connecting the grey matter "station" in the thalamus of the brain and the primary auditory cortex, where sound is processed primarily. A mask to identify the acoustic radiation was developed on MRI scans of a healthy population that had taken part in a large MRI study. The mask of the acoustic radiation was tested on our group with unilateral ear canal atresia, and normal hearing participants. It was equally hard to define the acoustic radiation in controls as in participants with atresia. We measured a parameter, called fractional anisotropy, on several places along the white matter tract, and no differences between participants with atresia and controls could be detected.

The primary auditory cortex was measured in the third study. Grey matter is where the cell bodies of the brain are located. The grey matter of the auditory cortex was defined and measured from the participants MRI scans. The auditory cortex of the left side was thinner than the right side in atresia, while the thickness is symmetric in normal hearing controls. The volume and total thickness were not different from normal hearing controls. It is not known if the difference in symmetry of the thickness that was shown in atresia is linked to functional deficits, although we speculate that it might be linked to language function, which is a left sided dominant function in most humans. The asymmetry pattern was not reversed in right sided versus left sided atresia, and the level of the hearing loss was not related to the thickness.

A rat model of atresia was used in the fourth study. Rats have larger "stations" of grey matter under the cortex of the brain, and there is a long tradition in studying the auditory structures in rats. Rats start to hear at day 15, so ear canal of the left ear was removed before that through surgery under anesthesia. The rats lived for 12 months and studied several times with MRI scans during their life. The rats were then euthanized, and the brains scanned for several hours in a very strong magnetic field scanner. This allowed for the images to be very clear. The white matter tracts of the whole brain and more specifically the bundles known to be involved in hearing processing were examined. We found stronger connections between two relay stations (from the cochlear nucleus to the inferior colliculus) on the hearing side, and weaker on the side of the hearing loss. This finding is supported by a previous study on ferrets with single sided hearing loss, using another method. We found that the measurement fractional anisotropy, was higher in rats with left-sided hearing loss than in normal hearing control rats. This could mean that the white matter has fewer crossings with other white matter bundles or is thicker and more organized in rats with atresia, although the reason is not certain because there was unfortunately a difference in preservation time before scanning.

Abstract

The following thesis aims to give more insight into the functional and structural response of the central auditory system to congenital unilateral ear canal atresia (UCA) and the accompanying asymmetric hearing with conductive hearing loss on the atretic side.

There is clear evidence that unilateral hearing loss, including UCA, has a negative impact on sound localization ability and perception of speech in noise. There is a spread in performance within the group, and the reason for this is not well known. In **paper I** of this thesis we examined sound localization with eye tracking and perception of speech in a cocktail party setting, in participants with congenital unilateral ear canal atresia, who had no hearing aids before age 12 (n=12) and compared to normal hearing references. Results show that the level of hearing loss on the atretic ear was associated with sound localization ability but not to speech perception.

In the second study, participants with UCA (n=17) underwent MRI-scanning of the brain with diffusion weighted imaging (DWI). A method is described how to segment the white matter bundle between the medial geniculate body of the thalamus and the primary auditory cortex, the acoustic radiation (AR). Methods to define the AR are previously described in high resolution diffusion weighted imaging (DWI) scans but is very time consuming or has problems with including more structures around the primary auditory cortex (PAC). An algorithm was trained to quickly segment the core of the AR in individual clinical scans. The white matter tract was also assessed with measurements of fractional anisotropy (FA), but no differences were found between UCA and normal hearing (NH) controls.

The third study describes the measurements of the grey matter of the primary auditory cortex of the Heschl's gyrus in the same participants as in **paper II**. Thickness and volume of the Heschl's gyrus were compared within the groups of UCA and controls, and between the groups. A difference in thickness was found between the left and right side (right thicker than left, corrected p=0.0012) in UCA, whereas controls had symmetric thickness. Volumes and total thickness were not different compared to controls.

Rat brains from 12 months old rats with a surgically constructed left-sided ear canal atresia were examined in **study IV**. DWI was acquired in a research camera

for rodents, 9.4 T magnetic field and a prolonged scanning time. Tractography and FA measurements were obtained both from whole brains and from tracts between auditory regions of interest (ROIs) using two different software. FA was generally higher in UCA rats than in controls. The AR was asymmetric in FA (left<right) in UCA, whereas FA was symmetric in controls. The FA was found to be lower at the left connection (same side as hearing loss) cochlear nucleus -inferior colliculus compared to the right side in UCA, while it was symmetric in controls. This finding (CN-IC) aligns with previous histology findings in ferrets with unilateral conductive hearing loss.

List of scientific papers

The papers included in the thesis are listed below and will be referred to by the numbering indicated to the left.

I. **Siegbahn M**, Engmér Berglin C, Hultcrantz M, Asp F. (2021). Adults with unilateral congenital ear canal atresia -sound localization ability and speech in competing speech in unaided condition. Acta Otolaryngol. 2021 Jul;141(7):689-694.

II. **Siegbahn M**, Engmér Berglin C, Moreno R. (2022). Automatic segmentation of the core of the acoustic radiation in humans. Front. Neurol. 2022 Sep 23, 13: 934650.

III. **Siegbahn M**, Jörgens D, Aspp F, Hultcrantz M, Moreno R, Engmér Berglin C. Asymmetry in cortical thickness of the Heschl's gyrus in unilateral ear canal atresia.
Accepted for publication in Otology & Neurotology, 2023 Oct 07.

IV. **Siegbahn M**, Kraft S, Maschio F, Hultcrantz M, Engmér Berglin C, Moreno R. White matter tracts of the auditory pathways in experimental unilateral ear canal atresia.
Manuscript.

Contents

List of abbreviations

AI	Primary auditory area (rats)
AC	Auditory Cortex
AF	Arcuate fasciculus
AR	Acoustic radiation
BCD	Bone conduction device
CN	Cochlear Nucleus
CSD	Constrained spherical deconvolution
DWI	Diffusion weighted imaging
HCP	Human connectome project
HG	Heschl's gyrus
IC	Inferior colliculus
MG	Medial geniculate body
MRI	Magnetic resonance imaging
NMR	Nuclear magnetic resonance
OR	Optic radiation
PAC	Primary auditory cortex
PTA4	Pure tone average (500, 1000, 2000, 4000 Hz)
SIFT	Spherical constrained deconvolution filtering of artifacts
SO	Superior olivary complex
SSD	Sudden sensorineural deafness
STG	Superior temporal gyrus
UCA	Unilateral congenital ear canal atresia
PP	Planum Polare
PT	Planum temporale
UCHL	Unilateral conductive hearing loss
UHL	Unilateral hearing loss

Introduction

Congenital unilateral ear canal atresia (UCA) is a relatively rare malformation affecting 0.83-17.4 per 10.000 births and is more commonly a unilateral condition [1,2]. The atretic ear with an atretic plate instead of an external ear canal causes severe conductive hearing loss[3,4]. Asymmetric hearing leads to difficulties in sound localization[5–10] and understanding of speech in a noisy environment[11–13].

These reported deficits are certainly a handicap in daily life to some individuals, however not to all[14]. It has been debated whether the worse measurements of the affected ear reflect needs of hearing aids, and if there is a therapeutic window in childhood when the patient needs to be fitted with a bone conduction device so the central auditory pathways can adapt to it. The resemblance with amblyopia and cortical ocular representation has been made[15]. A critical period in early childhood is described when the auditory system develops and most of the signaling in the brain is excitatory and later switches over to a predominantly inhibitory signaling phase. During the early period, the brain is more prone to plastic changes[16]. In allegory with early CI in congenital deafness for optimal language development[17], the question of early bone conduction device for unilateral atresia has been a topic, although there is already one hearing ear. In rats, the primary auditory cortex develops its tonotopic organization very early in life[18] and unilateral conductive hearing loss has been shown to interfere with this development[19].

This thesis aims to describe possible functional and structural changes related to adaptation to asymmetric hearing during sensitive years in childhood.

In **study I**, adult participants with UCA and no hearing rehabilitation in childhood were assessed in unaided condition: functional auditory measures were made in simulated natural daily life situations. Speech discrimination and sound localization were compared to pure tone audiometry thresholds. This study investigates if subjects with UCA eventually adapt, and if the hearing thresholds can give a hint to who will have a better functional hearing without hearing aids.

In **study II and III**, a cohort of adult participants with UCA with no hearing habilitation in childhood is examined with MRI of the brain. Structures of the auditory pathways are defined and compared to normal hearing, age- and gender matched controls. To our knowledge there are no published studies of the morphology of the central auditory pathways in humans with congenital

unilateral conductive hearing loss as in atresia. There is a recent functional resting state MRI study showing no large-scale network differences but a left sided auditory "small world" connection (nodal betweenness) deficit in right sided atresia[13]. In acquired unilateral hearing loss, the primary auditory cortex of the Heschl's gyrus (HG) has been studied with MRI, with findings like decreased volume of the right HG [20,21] and a correlation was found in bilateral sensorineural hearing loss with thickness of the left HG and hearing thresholds[22]. Although there is no golden standard in MRI measurement techniques, an attempt is made to compare our results with previous findings.

The assessment of white matter bundles *in vivo* is certainly a challenge. Especially in the auditory system where bundles are smaller, a lot of left-right hemisphere crossings occur and larger white matter bundles pass closeby[23,24]. A refined method is described in this thesis, in **study II**, for application in clinical scans to define the white matter bundle of the acoustic radiation with diffusion weighted (DWI) MRI. DWI is also used to examine auditory white matter bundles in a rat model with UCA, in **study IV**. Rats have proportionally larger subcortical auditory structures, which facilitates the investigation of subcortical white matter bundles between them. The aim of **study IV** was to compare an experimental model of UCA with normal hearing controls, to find out if auditory white matter tracts are different in UCA.

1 Literature review

1.1 Congenital atresia of the ear canal

Absence of an external auditory canal from birth, congenital ear canal atresia, with or without microtia, is a relatively rare malformation with an incidence of approximately 0,83-17,4 in 10,000 births[1]. Incidence in a Swedish population is reported to 2,4 in 10,000 births[2], which corresponds to an incidence of 25 cases yearly in Sweden. There is a male predominance, 57-67 % are right ears, and a majority, 79-93% is unilateral[1]. The malformation occurs either in isolation or as part of a syndrome, i.e. Treacher Collin's, Goldenhaar or CHARGE. A syndromic background is more common in bilateral cases[25]. The mechanism is thought to be a disturbed migration of the first two branchial arches in the developing fetus with increased cell death at gestational day 22-23[26]. The mandibula and the six loops of Hillock are constituted from the first and second branchial arch. The external ear in turn, is formed from the six loops of Hillock. The external auditory canal is derived from the first pharyngeal cleft. In atresia, os tympanicum, a part of the temporal bone, is missing, and instead there is an atresia plate adjacent to the commonly smaller middle ear. The ossicles are often malformed including a complex formed by malformed malleus-incus, and facial nerve anomalies are common[27], see **figure 1**.

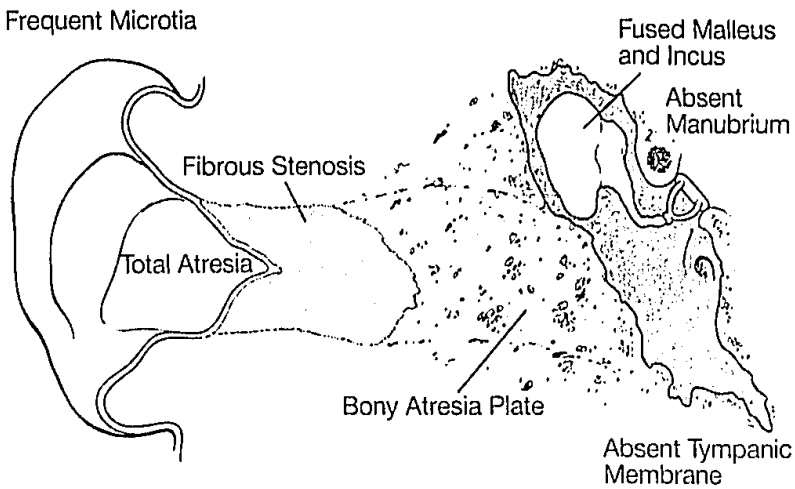

Figure 1. Drawing of ear canal atresia by [27], reprinted with permission from the journal.

1.1.1 Hearing in ear canal atresia

Hearing thresholds in congenital ear canal atresia are reported to be around 65-70 dB HL (PTA4), with an air bone gap of 43-58 dB[3,4]. Inner ear anomalies are reported in these studies to be present, more common in syndromic cases than in non-syndromic cases, and often associated with facial nerve dysfunction. In a case series of 70 patients with non-syndromic UCA, Halle et al reported oval window abnormalities in 21%, round window abnormalities in 7% and inner ear dysplasia in 3% of the cases[28]. Bone conduction in atresia was analyzed in a study by Zhang et. al. (2016), where subnormal bone conduction was found with a Carhart's notch in 33 % of the atretic ears. The authors ascribe the higher bone conduction thresholds mainly to fixation of the ossicular chain[4]. Priwin et. al. reported significantly higher thresholds of the atretic ear in 20 % of cases with UCA, comparing bone conduction thresholds (pure tone average, PTA4: 500, 1000, 200 and 4000 Hz) of the atretic ear with air conduction thresholds of the contralateral normal ear[29].

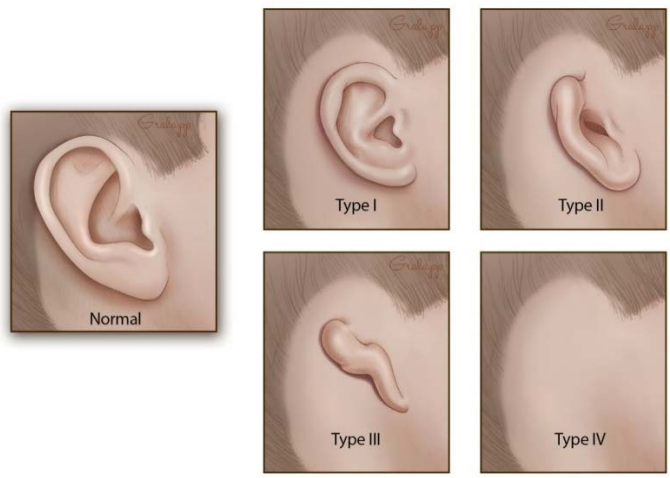

Figure 1. The four degrees of microtia. Type III and IV are usually associated with complete absence of the ear canal, atresia. Grade III is the most common, and grade IV is only observed in 6% [25]. Image with permission from Professor Chang reprinted from [30].

1.2 Unilateral hearing loss, consequences for speech and education

Studies on unilateral hearing loss (UHL) including both conductive- and sensorineural hearing loss has reported association with social consequences

such as increased use of remedial education, and an increased need (22-35%) to repeat a grade in school[31]. Children with UHL more often have a delayed language development[32]. A cohort study of 40 unilateral atresia patients, showed that none of their sample repeated a grade, but 65% needed special resources in school, including 47,5 % having an individualized education program and 45% used speech therapy[33,34]. Jensen et al. reported needs of remedial education and speech therapy in the same range[35] although these studies were criticized in a metanalysis[14] for the high risk of bias because of the selection of participants from centers where parents go because their children might be in a bigger need for assistance with their condition, and the studies were based on parental reports. Based on this, the question remains how large the consequences are concerning academic performance for children with unilateral atresia.

The ability to extract speech in a noisy environment is deteriorated in patients with UHL, including UCA[11–13]. The ability to distinguish target source from other interfering sound sources, the phenomenon known as spatial release from masking, is dependent on two ears[36]. Speech perception in noise was worse than expected when presented to the atretic ear in UCA[29].

1.3 Sound localization ability in unilateral hearing loss

Binaural hearing is crucial for accurate sound localization in the horizontal plane. UHL has been shown in several studies to bear major impact on sound localization ability in both children[5,6] and adults[7–10].

Binaural cues to localize sound source are composed of interaural time differences, and interaural level differences. Interaural level differences is a combination of spectral shape cues composed of the shape of the pinna, and the head shadow effect[36]. In vertical and front back localization, subjects rely on spectral shape cues, with help from the shape of the pinna[37,38,39,40]. For localization in the horizontal plane (azimuth) subjects depend more on interaural level and time differences. Binaural localization by interaural phase differences, is a more certain cue for lower frequencies[36]. For frequencies above 1700 Hz, wavelengths that are less than the distance between the two ears, the interaural level difference using the head shadow effect, becomes more important. Viehweg and Campbell (1960) found that in conductive unilateral hearing loss, the size of the error was not as big as in sensorineural loss[6]. Localization was helped by elevation of the hearing level of the sound source in conductive loss,

indicating that even a few audible sounds reaching the impaired ear can help for localization [41,42].

1.4 Ascending auditory pathways

1.4.1 From ear to cortex

In this part of the chapter, the journey of the sound from pressure waves in the air to cortical action potential and our awareness is summarized shortly.

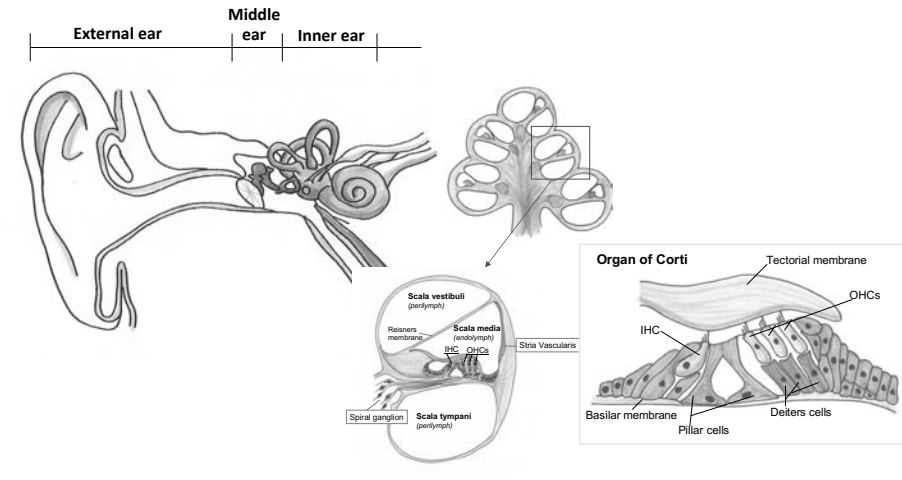

Figure 2. The normal human ear. A transection of the cochlea is shown at the bottom right with the organ of corti. Illustration by Annika Elmqvist Stenberg and Rusana Bark, Karolinska Institutet, with permission. In ear canal atresia, the external ear canal is missing, and the ossicles are commonly malformed.

In the normal ear, sound pressure waves from the air comes through the external ear canal, reaches the eardrum that starts to vibrate and transduce sound through the ossicles to the fluid filled cochlea of the inner ear. The vibration in the cochlea moves the basilar- and tectorial membranes, which triggers the sensory cells of the ear, the inner hair cells. Ion channels open in these cells releasing an action potential that transduces as a nerve signal from ganglion cells of the cochlea through the VIII cranial nerve to the first relay station, the cochlear nucleus (CN) in the brain stem. After this, the signal goes to the superior olivary complex (SO), both left and right side, the lateral lemniscus (LL), the inferior colliculus (IC), the medial geniculate body (MG) of the thalamus and then through the white matter tract the acoustic radiation (AR), to the primary auditory cortex (PAC). PAC is located in the transverse temporal gyrus, or the

Heschl's gyrus (HG). The signal crosses over from left to right and vice versa at several levels from brain stem to cortex. The overcrossing signal from the CN, to the SO as well as to the IC is important for sound source localization (comparing ipsi- and contralateral signal), both through the difference in sound intensity and in time[43,44]. From the IC, the signal is passed on to the MG of the thalamus. In normal hearing subjects, most of the signal from the left ear will finally reach the right cortex and signals from the right ear the left cortex[45], this is further described under the heading **1.4.4** cortical sound processing. A schematic figure of the rat central auditory system, **Figure 5**, which is very similar to human, is added under **1.6** rat model for hearing research.

1.4.2 The acoustic radiation

The white matter tract connecting the medial geniculate body of the thalamus with the auditory cortex is called the acoustic radiation[46]. The core of the acoustic radiation reaches the Heschl's gyrus (HG), and the fan connects the superior temporal gyrus where the secondary auditory areas are, as well as social-emotional integration[47]. As found in studies on animals (monkeys, rats and mice) the ventral MG is tonotopically organized and projects to the PAC, which is also tonotopically organized [48-50], suggesting that the fibers of the core of the acoustic radiation also have a tonotopic organization. Maffei et al. (2018) developed an atlas to define the acoustic radiation in human subjects, based on diffusion weighted imaging, controlled for by post-mortem dissection[51]. Another description with post-mortem brains as subjects also exists, by Bürgel et al (2006) tractography mapping with an atlas of the acoustic radiation and histological examination. They comment that there is a large variability in anatomy, and atlases are not subject specific. Interhemispheric anatomical differences in the AR have also been documented in human post-mortem brain specimens, with a more anteriorly located AR on the right than the left side, and a large variability in volume of the AR between individuals[52]. Two association fiber bundles are in close relation to the acoustic radiation: the inferior occipito-frontal and uncinate fascicles[53]. The uncinate fasciculus connects the medial- and lateral orbitofrontal cortex with the anterior temporal lobe, and has a role in associating known persons with images[54] or with voices and emotional memories[55]. The fronto-occipital fascicle exhibited anomalies in schizophrenia patients with auditory-verbal hallucinations[56], suggesting this bundle also has a role in voice- or sound recognition. Another three bundles are described to be in close relation to the HG: the arcuate fasciculus, the middle longitudinal fasciculus and the optic radiation[24]. With Tractseg[57], the white matter tracts of the human

brain including the AR, can be identified specifically per subject based on diffusion data. However, the existing mask of the AR also includes the STG.

1.4.3 Primary auditory cortex

PAC occupies most of the HG, or the transverse temporal gyrus, of the temporal lobe. Brodmann defined area 41 in 1909, a koniocortical area, which occupies most of Heschl's gyrus. Brodmann area (BA) 41 is adjacent to BA 42 caudolaterally, BA 22 rostrolaterally and BA 52 medially. The auditory association cortices, corresponding to BA 42 and 22, are the planum temporale (PT) and planum polare (PP). PT and PP constitutes parts of the superior temporal gyrus[58]. Primary auditory cortex receives input from the medial geniculate complex of the thalamus. Nerve fibers of the auditory tracts cross over to the contralateral hemisphere on brainstem level, but fibers also pass ipsilaterally without crossing [59].

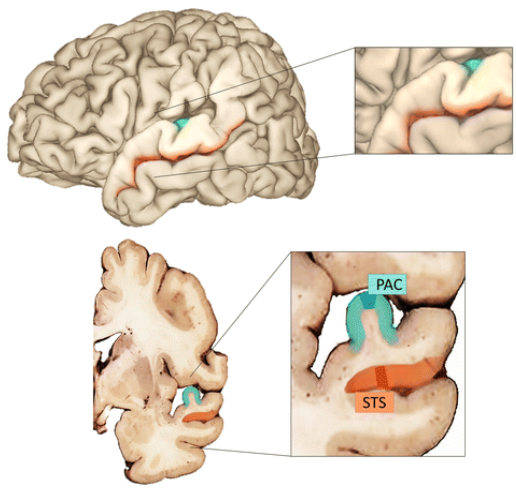

Figure 3. The primary auditory cortex (PAC) of the Heschl's gyrus marked in green. The superior temporal sulcus (STS) is marked in red. Illustration from Wikimedia commons, Bradley P. Ander, Nicole Barger, Boryana Stamova, Frank R. Sharp and Cynthia M. Schumann, 19th June 2015.

1.4.4 Cortical sound processing

To understand how cortical sound processing is affected in UHL, basic knowledge about normal sound processing is important. Primary auditory cortex (PAC) has a tonotopic organisation[60,61]. Sound is not equally processed in the right and left PAC. The lateralization on cortical level processing of sound is partly dependent on sound modality, not only receiving ear, where music like

sound is processed by right hemisphere and word like sounds by the left[45,62]. The dichotic listening test is a psychoacoustic test where word like- or other sounds are presented to both ears simultaneously to determine psychoacoustic attention and discrimination ability. This test can be used to decide preferred listening ear. The right ear is in most normal hearing cases the preferred listening ear for word like sounds[63], and the left ear for non-speech sounds[64]. The preferred listening ear is better at discriminating short term changes of signal in noise[36]. The right-ear listening advantage is not always concordant with handedness, as shown in a large study examining 373 healthy young adults, where 74% of all dextrals and 65 % of sinistrals were right-eared[65]. Morosan et. al. studied post-mortems brains with MRI and cytoarchitectural analysis. The hearing and the handedness of the subjects were unknown. The study show that there was a tendency of a larger Heschl's gyrus of left hemisphere than the right[66], which confirm findings of the studies by von Economo and Horn in 1925 (reviewed by[67]). Furthermore, another cytoarchitectonic study of post-mortem brains shows an increase in cell number of the left auditory association cortex, PT, compared to the right side, which reflects left hemisphere language dominance[68].

1.4.5 Cortical sound processing in unilateral hearing loss

In patients with UHL, studies have shown auditory processing to be altered. While normal hearing subjects process sound predominantly contralaterally to the receiving ear, unilateral hearing loss subjects tend to process sound reaching the normal ear more evenly distributed between hemispheres, as measured by task-based fMRI and electrophysiological methods [59,69,70]. Parry et. al. 2017 measured cortical auditory evoked potentials (CAEPs), in adult patients with chronic, conductive UHL as a response to sound presented via bone conduction to the hearing-impaired ear. They found significantly larger amplitudes from auditory cortex than in NH. This supports that unilateral sound deprivation causes increased responsiveness in the central auditory system[69]. On the other hand, Vasama et al. recorded CAEPs in six participants with congenital unilateral conductive hearing loss, aged 7-28 years: There was a spread in results, but most participants had amplitudes and latencies within the normal range[71], however the sample size was very small. Pross et al 2015 examined 12 adults with single sided deafness (SSD), and 12 NH controls with magneto encephalographic imaging (MEGI) and MRI with repeated tone burst stimuli to each hearing ear. In SSD subjects there was a statistically significant interhemispheric latency difference, with a reduction of latency time in SSD compared to NH group. The authors conclude that the ipsilateral cortex to the deaf ear is under-stimulated. They also speculate that the missing ipsilateral inhibition from the deaf ear in

stimulating the hearing ear may lead to these results and is a sign of cortical plasticity[72]. In an fMRI study of processing of speech in noise in UHL subjects, right sided hearing loss subjects show more pronounced differences in activation of defined regions of interest (ROI) in speech processing compared to NH subjects[73]. This study considered adult-onset hearing loss, sudden idiopathic deafness, and possibly reflects the effect of the above-described preferred listening ear, with better discrimination of language when listening with the right ear in most cases and a more pronounced difficulty in this task when the right ear is affected by hearing loss. However, there is evidence that congenital right ear sensorineural deafness with normal hearing left ear still have a dominance of language function in the left hemisphere[74], although it is not very clear if the left PAC is specialized as in NH subjects for rate related changes. In rats, early onset UCHL gave disrupted tonotopy pattern of the PAC[19].

1.5 Imaging of the central auditory pathways

1.5.1 Magnetic resonance imaging

Paul Christian Lauterbur (first published in Nature 1973[75]) and Peter Mansfield[76] shared the Nobel prize in medicine 2003 for their discoveries that the physical phenomenon nuclear magnetic resonance could be used to create images from spatial information of the signal, known as magnetic resonance imaging (MRI)[77].

Nuclear magnetic resonance is a physical phenomenon of the protons having magnetic properties: Protons in a strong magnetic field will align with it and spin at a specific frequency, which is referred to as the Larmor frequency. A radiofrequency pulse can make them turn perpendicular to, or misalign with the main external magnetic field. After excitation by the pulse, eventually the protons fall back to their initial state while emitting a weak signal that is recorded with coils placed close to the imaged body. The spatial encoding is obtained through a magnetic gradient. The signal has slightly different properties depending on the chemical environment surrounding the proton. The recorded signal, with different frequencies and phases, is then transformed through the mathematical operation, Fast Fourier transform, to create an image in the spatial domain as different contrast (signals) in voxels.

Since the human body is filled with protons in hydrogen atoms: in water, proteins etc., the technique is excellent to create images for diagnostic purposes especially in soft tissues. The most well-known contrasts used are T1 and T2

weighted imaging, the T1 relaxation (spin-lattice relaxation time) is related to the time protons need to realign with the external magnetic field. T2 (transverse relaxation time) is related to the time for the spins to dephase in-plane after excitation.

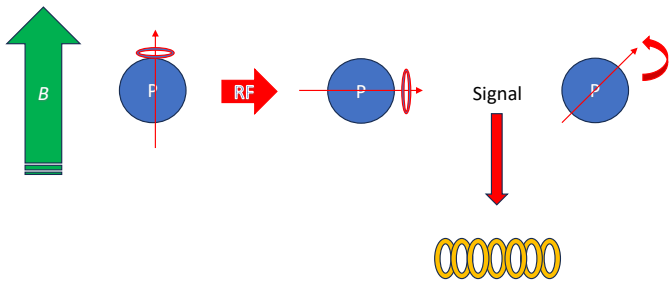

Figure 4. A simplified explanation of the physics behind MRI. *B*= main magnetic field, P= proton, RF= radiofrequency pulse (90 degrees in this example), arrows and circles illustrate the spin of the proton and alignment to the magnetic field. The signal is measured at the coil (in yellow).

1.5.2 Diffusion-weighted imaging

In **studies II and IV**, diffusion weighted magnetic resonance imaging (DWI) and probabilistic tractography, were used to track white matter fiber bundles of the acoustic radiation in human participants with UCA and controls, and to assess white matter bundles for whole brains in rats with UCA and controls.

DWI uses the signal from water diffusing along myelinated axons to track and assess the quality and orientation of white matter bundles[78]. Water diffuses in different directions over time, also known as Brownian motion. If the diffusion is the same in all directions, it is called isotropic diffusion. If the diffusion is *not* the same in all directions, it's called anisotropic diffusion. The diffusion of water in grey matter of the brain is almost isotropic, whereas it is anisotropic in white matter. DWI makes it possible to study white matter *in vivo*. Anisotropy in white matter can be measured as e.g. fractional anisotropy (FA)[79]. The FA is computed as

$$FA = \frac{1}{\sqrt{2}} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

and can be interpreted the magnitude of diffusion in the major direction (eigenvector 1, eigenvalue λ1) compared to the two orthogonal directions (λ2 and λ3). FA can decrease in presence of crossing tracts within a voxel or a region, or if white matter bundles are injured or demyelinated.[80] FA=0 indicates isotropic diffusion[81].

DWI is used in clinic to assess white matter in a wide variety of neurological disorders, e.g., stroke[82], cerebral palsy[83], neurodegenerative diseases, brain tumors and traumatic brain injuries[84]. The technique is also used in other tissues, e.g., in ear diseases in evaluation of recurrence or residual of cholesteatoma disease[85].

Tractography can be used to track the diffusion vector in voxels along axons, recreating white matter bundles from one region of interest to another. Farquharson et al (2013) draw the conclusion that both deterministic and probabilistic fiber tracking underestimates the tracts, and that constrained spherical deconvolution (CSD) improves the results. The best combination was probabilistic fiber tracking and CSD[86]. The same results were found in cranial nerves[87].

Zanin et al (2019) discusses in a review the difficulties of mapping the auditory white matter tracts; the closeness to other, larger white matter bundles and the off-resonance distortions from e.g., the air in the ear canal close to the brainstem[23]. The acoustic radiation connecting the MG and the PAC of the Heschl's gyrus (HG) is challenging to track due to its closeness to the Arcuate fasciculus (AF), Optic radiation (OR) and the Middle longitudinal fasciculus (MLF)[24].

Ideally, Diffusion weighted imaging should have high spatial and angular resolution. To correctly identify crossing fibers, data needs to be acquired at several angles which comes at the cost of an increased scanning time. Spatial resolution and contrast are also very important, as discussed by Ford et. al.[88] The subcortical nuclei require sufficient contrast for correct identification and for this higher field power MRI-scanners (e.g. 7 T) might be required, to be compared with the 1,5 or 3 T that are used in clinical practice. However, high-field MRI on the other hand, induces image distortions due to chemical shift artefacts, increased magnetic field inhomogeneities and eddy currents to a greater extent. There are several synapses close to, and as a part of the

auditory pathway, especially at brainstem level, which is a challenge of tractography algorithms to correctly identify, and reconstruct white matter pathways around[23].

1.6 Rat model of hearing loss

The rat is a traditional animal model to study the auditory system, because it shares many important features with humans such as general anatomy, physiology, and behavioral response to sound[89]. The subcortical auditory nuclei are more feasible to study, due to their proportionally larger size compared to human structures [90]. Further, a surgical procedure to simulate congenital conductive hearing loss, is possible due to later hearing onset in rats. A study by Geal-Dor et. al. 1993, where hearing thresholds were tested in rat pups by ABR-response, air conduction thresholds were measurable at 80 dB, postnatal day 11. The rats reached adult thresholds at postnatal day 22[91].

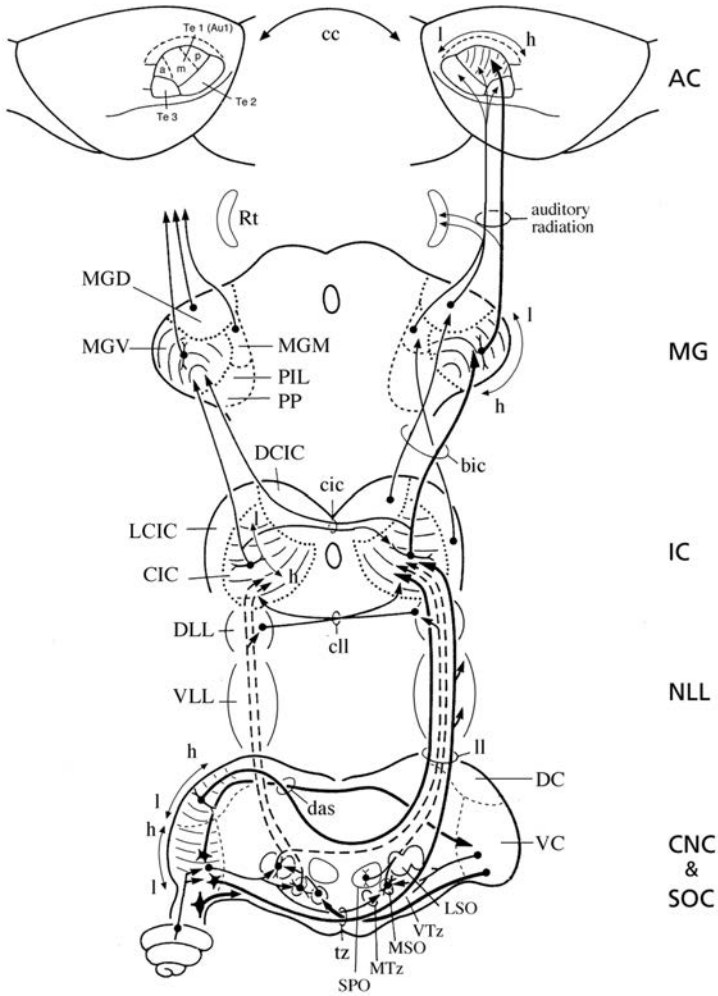

Figure 5. Overview of the rat central auditory pathway, which is similar to human. Illustration with permission from Professor Malmierca, modified from the originally published version in[89]. To the right abbreviations: AC= Auditory cortex, MG= Medial geniculate complex, IC= Inferior colliculus, NLL= Nuclei of the lateral lemniscus, CNC= Cochlear nucleus complex, SOC= Superior olivary complex. Note the auditory (or acoustic) radiation connecting the MG and the AC. In the image, letters are indicating e.g. M=medial, L=lateral, C=central, V=ventral, D=dorsal, as a specification on the parts of the nuclear complexes.

1.7 Morphology and resting state functional magnetic resonance imaging of auditory cortex and acoustic radiation in hearing loss

Studies of cortex morphology in subjects with UHL and bilateral hearing loss have been conducted with a variety of techniques with diverging results. The variety in technique and outcome measure make comparisons challenging. As a reference, in **study III** of the present thesis, Freesurfer was used to measure

cortical grey matter volume and thickness[92]. Histological measurement on 27 specimens obtained from neurosurgery compared to presurgery MRI measurements with Freesurfer, show high reliability of Freesurfer with a cortical thickness of 3.65 + /- 0.44 mm as compared to histological measurements of 3.72 +/ - 0.36 mm (p=0.32).

Wang et. al., examined grey matter volume with voxel-based morphometry in 42 participants with UHL caused by schwannoma and 24 NH controls. They found a grey matter volume decrease in primary auditory cortex (Heschl's gyrus) of the right side irrespective of side of hearing loss, a decrease of volume of visual (calcarine) cortex and increase in somatosensory and motor cortex. Contralateral to side of hearing loss, there was a volume decrease in perirhinal cortex[20]. Rueckriegel et. al. examined UHL caused by vestibular schwannoma with DTI and volumetric measurements of the acoustic radiation in 15 patients (8 left side and 7 right side), no NH controls were examined for comparison. They found that volumes of the acoustic radiation were negatively affected on the contralateral side to the hearing loss ear, although in five out of 15 participants the acoustic radiation was not completely tracked. The fractional anisotropy was not different[93]. In two studies of prelingual bilateral deafness, no differences were observed of grey matter volumes of the PAC of deaf adult subjects compared to NH. Broca's area was found to be larger in deaf subjects in one of these studies[94], and the other study found left motor hand area of cortex to be larger in deaf, signing, subjects[95].

A study on children with right-sided UCA (n=27) and matched controls, were examined with rs-fMRI and functional hearing tests: audiometry and speech understanding. No large-scale resting state networks were found different, although small world networks as measured with nodal betweenness (NBi), show decreased connectivity in the left superior temporal gyrus and the attention network, and higher NBi in visual field and dorsal mode network. The authors speculate this might indicate a compensatory sensory mechanism[13].

A cohort of children with sensorineural UHL, were examined with diffusion weighted imaging (DWI) based tractography and rs-fMRI, and compared to normal hearing siblings, in two different studies[96,97]. The study concerning rs-fMRI found a stronger correlated activity in UHL group than in controls, between seeding ROI left inferior parietal lobule and posterior operculum region. The authors speculate this is due to a need for more subvocal rehearsal, to maintain

work memory from auditory perceived instructions[96]. The authors do not comment on any changes within the primary and associative auditory areas. The other study on the same cohort, studying DWI and cortical morphology, found FA to be increased in the left lateral lemniscus of children with right-sided UHL, compared to left side UHL. Microstructures of auditory ROIs grey matter were found to be preserved compared to NH, including left-right asymmetry pattern. A correlation was found between measurements of white matter structure of the left Heschl's gyrus, i.e. the higher the FA value, the less likely the child was to need remedial education[97].

1.7.1 Animal studies in unilateral conductive hearing loss

Studies in the 1970-and 80s suggest that unilaterally induced conductive hearing loss (UCHL) in rats and mice can give altered numbers and / or sizes of the cell bodies of the cochlear nucleus (CN) on the side of hearing loss [98,99]. However, the findings were not replicated in ferrets: Moore et al (1989) found on the other hand, that their experiments in ferrets induced weaker projections from the CN to the inferior colliculus on the side of conductive hearing loss, compared to the contralateral, hearing side[100].

Popescu and Polley (2010) studied rats with unilaterally ligated ear canals, at postnatal 2 weeks, 4 weeks, or adult age. Tonotopy of the A1 (primary auditory cortex) was disrupted in rats ligated at 2 weeks but not at 4 weeks or adulthood. This suggests a sensitive period in early life for binaural integration and cortical organization[19]. The same conclusion was drawn by Liu et al (2021) who studied rats with UCHL with onset early (postnatal day 14-18) or later: that early onset UCHL gave less binaural interaction representation at A1 cortical level[101].

Tucci et al. [102] studied the central auditory system in gerbils with unilateral removed malleus, or unilateral cochlear ablation and a group with a sham procedure. The animals were either 21 days at the procedure or adults and lived for 3 weeks after the procedure. A radioactive glucose was administered, and the central nervous system was examined ex vivo. Gerbils have hearing onset at postnatal day 12. The experiment can be considered to study a loss of function rather than congenital asymmetric hearing. The IC contralateral to the impaired ear showed lower uptake of 2-deoxyglucose in young animals (hearing loss onset at day 21), equivalent to the effect found in cochlear ablation at the same age. A larger effect was seen in young animals than in adults.

The same authors examined the uptake of 2-deoxyglucose in the auditory cortex of gerbils [103]. UCHL was induced in 18 animals on day 21 and all were euthanized 3 weeks after the procedure. This study showed decreased uptake of glucose in the contralateral auditory cortex and confirmed decreased uptake of the contralateral IC, as seen in the earlier study.

1.8 Treatment in congenital unilateral ear canal atresia

For subjects with UCA, there are several available hearing aid solutions, and rarely, in selected cases, surgery with reconstruction of the external auditory canal, ear drum and ossicular chain to restore hearing. Auditory outcomes are reported to be generally better in treatment with hearing devices than with surgery, and more stable over time[104]. However, in a few cases with a mild form of malformation of the middle ear, and with a motivated patient, surgery might be an option. Hearing results can be predicted by the Jahrsdoerfer score system in these cases, after a temporal bone CT scan[105]. Active middle ear implant also requires more extensive surgery than other bone conduction implants; A score system, the aMEI score, has been developed to predict risks and facilitate decision-making using anatomical features and measurements on a CT scan[106]. Example of high-resolution CT-scan in UCA is shown in **figure 6**.

Compliance in usage of bone conduction devices (BCD) for this patient group is unfortunately low, as reported by Nelissen et al 2015[107]. Only 56% of patients were users after a mean follow up of 7 years after implantation. The study pointed towards younger subjects not using their implant, and the cause was reported disturbing background noise and too little benefit. This hypothesis can be strengthened by the study by Josefsson Dahlgren et al. where children fitted with a BCD (e.g. BAHA®, Ponto®) did not have significantly better sound localization or speech discrimination in competing speech setting[108]. Patients with UCA fitted with a BCD in adulthood, have less than expected understanding of speech in noise and less improvement than expected of sound localization [10,29,109]. The suggestion from Priwin and coauthors was that early auditory stimulation could facilitate central auditory speech processing. On the other hand, the younger subjects in the Nelissen et. al. study did not use their implants to a satisfactory extent as mentioned above. Hearing results of bone conduction devices are reported less beneficial in congenital cases than in acquired[10,109,110]. More side specific stimulation and less cross-over disturbance of the

contralateral ear are being assessed for this patient group, in the form of active acoustic implants, or active middle ear implants[111,112]. The active implants Bonebridge® and Osia® are FDA-approved from 12 years of age and approved for use in Europe from 5 years of age. The active implant Osia® is approved depending on the thickness of the skull bone (>3 mm). Implantation has been described from young ages[30]. Studies have shown promising results with better speech perception in noise with active middle ear implants (vibrant sound bridge®) with age at implantation already from 5 years[113–115] and in bone conduction device Bonebridge® with an increase in speech scores from around 30% to 98%[116]. The later study implanted children as young as 3 years of age. The need to consider other factors of hearing profile for each subject might be suggested to achieve better treatment results and adherence.

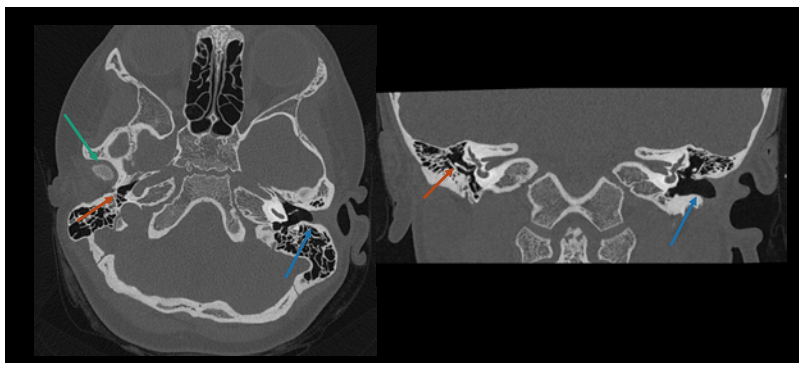

Figure 6. High resolution CT-scan of a seven-year-old girl with right-side unilateral ear canal atresia. Red arrow= atresia plate instead of ear canal. Contralateral ear, normal ear canal= blue arrow, green arrow= mandibular collum of the temporomandibular joint, in close relation to the underdeveloped middle ear.

2 Research aims

The aim of this thesis was primarily to widen the diagnostic possibilities for the central auditory pathways in UCA, beyond functional hearing tests traditionally used. Adult participants, both a rat model with UCA and human participants, were studied to find possible changes in the central auditory pathways related to adaptation to pronounced asymmetric hearing existing since birth.

Aims of **study I** were to find auditory markers that are possible to measure early in life, as hearing thresholds in audiometry, to correlate to functional hearing with speech perception and sound localization in adulthood. The aim was also to assess to what extent the participants with UCA who had not used hearing aids in childhood performed in complex auditory tasks.

Aims of **studies II and III** were to find possible structural changes of central auditory structures in MRI-scans of brains in UCA and controls.

In **study II** the white matter tract, the acoustic radiation (AR) between the primary auditory cortex of the Heschl's gyrus and medial geniculate body of the thalamus was examined with MRI as a possible marker in UCA patients, compared to normal hearing controls. During the research, methods needed to be developed to be possible to use in clinical MRI-scans, and the aim was therefore to create a mask for the core of the AR obtained through tractography on scans with higher spatial and angular resolution.

In **study III** the grey matter of the primary auditory cortex of the Heschl's gyrus was examined with MRI to find possible changes in UCA patients, compared to normal hearing age- and gender matched controls.

A translational rat model was used in **study IV** with the aim to find possible future targets of the central auditory pathways, because a prolonged MRI scanning time and higher magnetic field strength could be used and enhance image material to assess subcortical structures and white matter tracts between them.

3 Materials and methods

Table I. Overview the study design of the four studies included in the thesis.

	Study I	**Study II**	**Study III**	**Study IV**
Design	Cross-sectional study	Method description and case-control study	Case-control study	Experimental, cohort study
Studied group	Adults with UCA (n=12)	Adults with UCA (n=17) Matched controls (n=17) High reolution reference scans (HCP)	Adults with UCA (n=17) Matched controls (n=17)	Rat model with UCA (n=7) Control rats (n=4)
Outcome measures	Hearing thresholds, sound localization, speech perception	Tracking success and FA in acoustic radiation	Volume and thickness of the Heschl's gyrus, hearing thresholds	MRI diffusion data, FA of whole brain and between regions of interest
Statistical method	Student's independent t-test, Linear regression	Student's paired t-test	Student's paired t-test, Wilcoxon signed rank test, Linear regression	Student's paired t-test, Wilcoxon signed rank test, Wilcoxon matched pairs test

3.1 Study participants

In **study I, II and III**, participants with congenital unilateral ear canal atresia (UCA) were recruited from the Department of Otorhinolaryngology, and from a previous study[29]. For **study II** and **III** normal hearing healthy, age- and gender matched controls were recruited through the social network of the research group. Age inclusion criterion was set to 18-45 years of age, to lower the risk of undiagnosed neurologic disorders or effects on the brain of aging [117]. Participants were excluded if they had used a BCD before the age of 12, or if they had successful ear canal reconstruction with hearing thresholds <35 dB (for binaural hearing according to [118]. All patients and controls were asked if they had contraindications for MR-scanning, and a few had e.g., metal sutures from an external ear reconstruction, braces etc., and could only participate in the hearing tests, and another few participants did not want to participate in the extended hearing tests (localization and speech perception tasks). Patients were excluded if they had other conditions, or medications that could possibly interfere with the results (neuroleptic drugs, sever psychiatric or neurologic disorder, syndromes, contralateral hearing loss). A few subjects were eligible and participated in all the examinations offered, most of the participants completed either MRI-scanning and audiometry or extended audiometric testing. 18 participants with UCA and 18 age- and gender matched controls completed MRI scanning and audiometry. MRI-data was corrupt in one participant leaving 17 participants and 17 age- and gender matched controls to analyze. 12 participants with UCA completed audiometric measurements with localization task and speech in competing speech test, analyzed in **study I**.

In **study I**, NH controls (n=8) from a previous study[119] was used as reference. They were tested with the same test battery (pure tone audiometry, speech in competing speech and sound localization task) in NH condition, and with a unilateral earplug and muff, to create an experimental UHL with a mean of 43 dB hearing threshold, hence the name of the group UHL_{43}.

Material from the Human Connectome Project (HCP) was used in **study II**. The diffusion weighted scans of human brains of healthy volunteers from the HCP are publicly available for research. The HCP scans have a high spatial- and angular resolution due to a long scanning time in a research scanner[120]. The HCP

scans are commonly used as reference to compare clinical scans when new methods for tracking specific white matter bundles are developed.

Sprague-Dawley rats were used for **study IV**, view topic "rat model of atresia" section **3.2** below, for description of the creation of UCA model.

3.2 Rat model of atresia

To create a model of UCA, rats went through surgical closure of the left external ear canal, as described in two studies[121,122]. In a study by Kelly et. al. 1987, orientational response to sound stimuli was observed to be severely impaired in conductive UHL[121]. Further, this specific procedure used to close the ear canal, was associated with a low risk of cholesteatoma formation in rats[122]. Controls were kept and followed the same study protocol, except for initial ear canal surgery. The rats were allowed to live for 12 months and followed the study protocol according to **figure 7**.

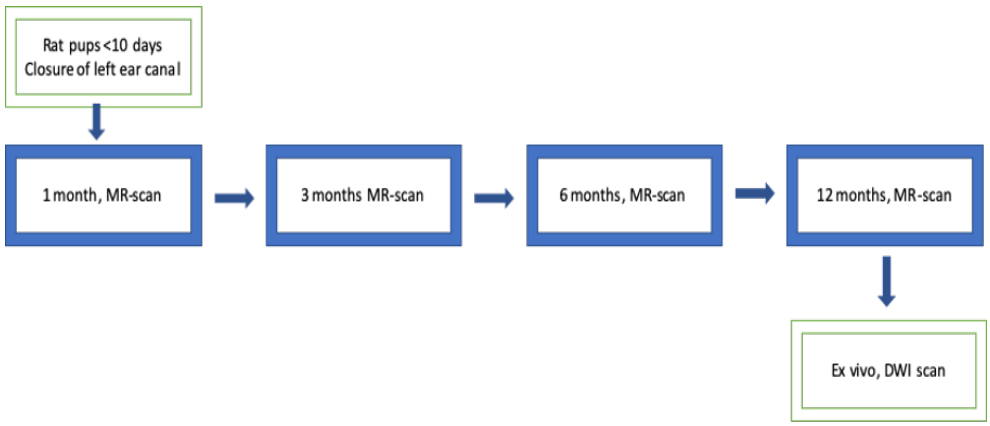

Figure 7. Study protocol for rats of **study IV** where the diffusion weighted data from the ex vivo scans are analyzed.

3.3 Magnetic resonance imaging

Basic physics of MRI is described under the introduction section **1.5.1**. Participants with UCA (n=18) and controls (n=18) were scanned in a 3 T GE Discovery MR750 3T MRI scanner for anatomical imaging (T1w and T2w flair), functional resting state MRI (not presented in the thesis) and diffusion weighted MRI. The data of 1 participant was corrupt, leaving 17 participants with UCA and 17 controls for analysis. In total, the imaging took 30 minutes to achieve. The

anatomical imaging was used to calculate cortical volume and thickness of the Heschl's gyrus where the PAC is located for **study III**, and the diffusion weighted imaging was used to track the core of the acoustic radiation in **study II**.

3.3.1 Diffusion weighted magnetic resonance imaging and processing of data

Diffusion-weighted imaging data (DWI) from a clinical protocol as described above under section **4.3**, was acquired with a clinical scanner (GE Discovery MR750 3T MRI scanner) with b= 1000 s/mm^2, 60 directions and spatial resolution 2.3 mm isotropic. In comparison, HCP dataset has 3 b-values (1000, 2000 and 3000 s/mm^2), and higher angular resolution with 90x3 directions and higher spatial resolution of 1.25 mm. It is much more challenging to track the AR in our dataset. The AR was tracked with a probabilistic tractography method using a tool in MRtrix3[123] on HCP scans. Filtering of tracts was done with constrained spherical deconvolution (CSD), estimating the fiber orientation per voxel[124]. Masks of the medial geniculate body and the Heschl's gyrus were automatically generated from Freesurfer[125] program and applied as seeds to start the tracking of the fibers, and the AR was tracked in both directions. From this information, the neural network TractSeg could be trained to create a mask of the core of the AR in individual cases based on diffusion data. The mask could be applied to both scans of the HCP and from our UCA participants and controls. For calculation and comparison of FA along the tract, the tracts were divided into several segments according to Yeatman et.al.[126] and Chandio et.al.[127] Success rate in defining the AR was compared, and the FA was compared between UCA and controls.

In **study IV**, rats were scanned *ex vivo*. Rats were sacrificed at 12 months of age through perfusion with normal saline. The heads were separated from the body, external ears and skin were removed. The skulls were kept in formaldehyde solution until scanning when they were put into an inert fatty solution (Fomblin®), to minimize off-distortion artifacts. The scanning time for the rat brains were several hours in a very strong magnetic field scanner (9.4 T compared to human scanners which are normally 1.5-3 T). Under these conditions, the diffusion weighted scans were optimized. Rat scans needed to be pre-processed, which means removing the skull bone from images and correcting artifacts due to eddy currents as a result of rapidly changing magnetic gradients. After this the regions of interest were manually marked on one control rat, the cochlear nucleus, superior olivary complex, inferior

colliculus, medial geniculate body and the primary auditory cortex (A1) bilaterally using straight forward visual comparison from a rat brain atlas[128]. The ROIs were then applied to the other subjects and adjusted to fit. Probabilistic tractography was performed using two different tracking programs. The flow chart in **figure 8** demonstrates the technical steps in pre-processing the image material and calculating the FA and the tractography procedure. Apart from using FSL for whole brain tractography and FA calculations, a separate analysis was made with the program MRtrix3, to calculate the FA between ROIs of filtered data. Tracts of interest were chosen: CN-IC and MG-AC and compared within groups left vs. right tract in UCA and controls.

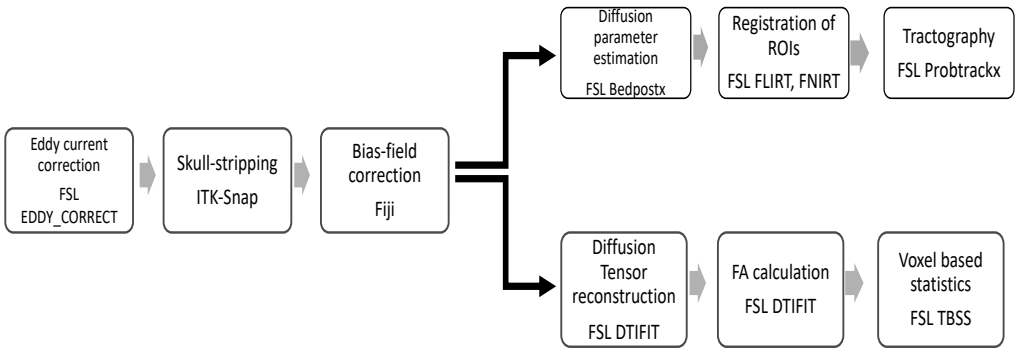

Figure 8. Flow chart of technical procedure in rat brain *ex vivo* study, where fractional anisotropy of whole brains was analyzed.

3.3.2 Cortical thickness and volume measurements

In **study III**, cortical thickness and volume measurements are presented and compared. T1 and T2w flair data were used to define regions of the brain with an automated, gyral based atlas[129]. Using the program FSL version 6.0, the grey matter of the brain was defined, and measurements of cortical thickness and volumes calculated. Cortical volume measurements were normalized to total intracranial volume as suggested by[130]. The choice was to consider only the Heschl's gyrus for analysis and comparison between UCA (n=17) and NH matched controls (n=17).

3.4 Hearing tests

3.4.1 Audiometry

Audiometry was used in study I, II and III to establish hearing level. Participants were seated in a soundproof booth, and hearing thresholds were set for air conduction (125, 250, 500, 750, 1000, 1500, 2000, 3000, 4000, 6000, 8000 Hz) and bone conduction (250, 500, 1000, 2000, 3000, 4000 Hz) whenever hearing threshold exceeded 20 dB HL. Masking of the contralateral ear was applied in participants with hearing loss.

3.4.2 Speech in competing speech

Hagerman sentences [131] with 5 words each were presented by a female voice from a loudspeaker in front of the participant. To simulate a cocktail party setting, four loudspeakers were placed around the participant according to **Figure 9**. The competing speech was presented at a fixed level at 63 dB sound pressure level (SPL), and the target speaker was adjusted to aim at 40 % recognition. Participants were instructed to not turn or tilt their head, monitored by the audiologist.

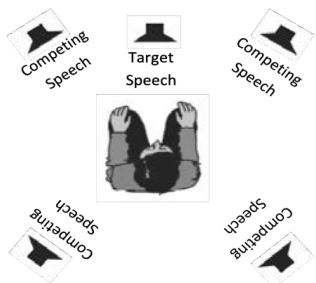

Figure 9. Setup for the speech in competing speech test. Image reused with permission by the creators Martin Eklöf and Filip Asp, Karolinska Institutet.

3.4.3 Sound localization test

The method used to measure sound localization is described in [132]. The participant was seated with a semi-circle of 12 loudspeaker-screen display (LD) pairs in front, -55 to +55 degrees, adjusted in height to azimuth with the head position according to **figure 10.** The test started at -5 degrees with sound stimuli, whereafter a movie was shown on the corresponding screen for visual

feedback. The sound- movie was shifted between the LD pairs 24 times in a pseudorandomized order. The speech weighted sound stimuli (musical doodle) were presented at 63 dB SPL. Participants were instructed to look for the sound source, and eye movements were tracked with a corneal reflex eye tracking device. Measurements were recorded during the last 500 ms of sound only period, and an angular error was calculated from the gaze angle and the LD pair position. An error index (EI) between 0-1, was estimated for each participant from the results, where 0 is perfect localization and 1 is chance performance. Calculation of EI is described in [132,133].

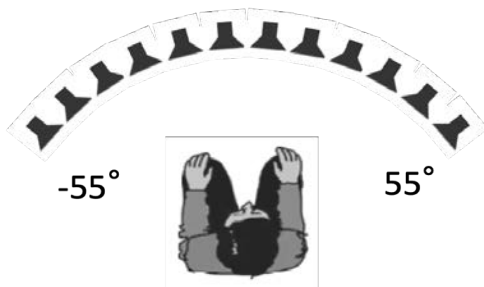

Figure 10. Setup for the sound localization task. Adjacent to every loudspeaker was a screen projecting a movie for visual feedback with a 1.6 seconds latency from sound switch. Image reused with permission by the creators Martin Eklöf and Filip Asp, Karolinska Institutet.

3.5 Statistics

Calculations of statistics were made in Statistica version 13 (Statsoft, Inc. Tulsa, OK) in **study I**, Tractseg tractometry tool Bundle Analytics (BUAN)[127] was used to compare FA values along the tract of the acoustic radiation in **study II** and Prism Graphpad version 9 (GraphPad Software, LLC) in **study III and IV**.

When comparing groups, if the parameters were normally distributed, a student's t-test was used. If not, or if the number of the group was too small to test (rats control group, n=4)), the Wilcoxon signed ranks test was used. In study II and III, participants with UCA and matched controls were compared with paired t-test. In study I where a reference group from previous study was used as controls, an independent t-test was used. In **studies I and III** regression analysis were also made, to look for correlations between pure tone average and cortical thickness, respectively.

Due to multiple statistical tests in **study II, III** and **IV** a correction of multiple comparisons was made using Bonferroni correction of significance level (*e.g.,* in

study III, 8 different categories tested, Bonferroni level of significance =0.005/8). Otherwise, a level of $p<0.05$ was considered significant.

3.6 Ethical Considerations

The participants included in this study have all signed an informed consent that made clear that it is possible to leave the study whenever they want to, without giving any specific reason. It was written in the information that MRI can reveal changes or disease that one is not aware of before the examination, some of which can require treatment. Fortunately, none of the participants in this study did have any en passant finding of clinical importance. MRI does not have any ionizing radiation, and not any known side effects.

Animal studies are of course requiring ethical reflection. It is important to keep in mind the purposes of the studies when conducting animal research. In this case the purpose was to find a diagnostic or predicting marker in unilateral congenital hearing loss. When considering research on animals, it is important to reflect over the three "R"s: Replacement, Refinement and Reduction. In the animal study of this thesis it was not possible to replace animal research with data models or cell cultures, because the purpose was to study the biology and plasticity of full brain networks. The aim was also to reduce the animals to the smallest number possible to be able to compare with statistics with reasonable power. There are several reasons why it is of value to study animals before or parallel to human subjects: The original study protocol contained studies over time with several MRI scans of the rats to follow possible changes in different stages of central nervous system development as a response to the hearing loss. The rats of course did not have any hearing aids, and today all children with atresia are offered hearing aids and the natural course of unilateral conductive hearing loss can therefore not be studied over time from childhood to adulthood. The rat life cycle is also much shorter which makes a longitudinal study feasible. There is a long tradition of studies on the central auditory pathways on rats which makes the research more comparable to earlier findings. There was also the possibility to use an MRI scanner for animal research purposes with much stronger magnetic fields (9.4 T instead of 1.5–3 which is in use for human subjects) and a prolonged scanning time to be able to acquire excellent data. The rats had a good living standard in enriched cages with free access to food and water and had anesthesia whenever an experiment or measurement was performed, including monitoring of their pulse, breathing, body temperature and were

awakened carefully in a separate, warm cage. In short, the suffering was minimized.

A part of the ethical consideration must also be the research not published from this project. There are a lot of examinations of animals that were not included in a paper yet. The methodological difficulties were too many to be certain of the results of the resting state functional MRI data. There was a dilemma on whether to publish results which were not reliable or leave it for later and the decision was not to include the data in this thesis.

4 Results

Study I

Results from 12 participants with UCA, with prospectively collected data was analyzed and compared to a normal hearing reference group from a previously published study, tested in normal hearing condition and with a unilateral earplug and muff constructing a unilateral hearing loss of mean 43 dB HL [119]. A correlation was found with linear regression of PTA4 (pure tone average, frequencies 500, 1000, 2000, 4000 Hz) of the atretic ear, shown in **figure 11**.

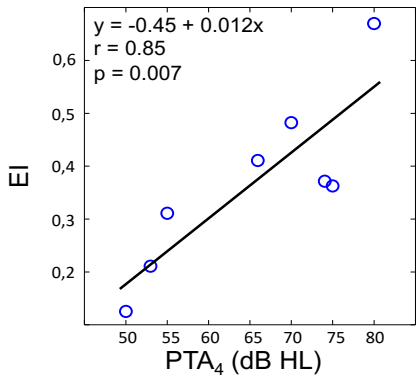

Figure 11. There was a correlation of sound localization accuracy and hearing threshold of the atretic ear (p=0.007, r=0.85). EI= error index, PTA_4= pure tone average of the atretic ear (air conduction). Figure republished from[134], with permission from the journal.

There was no correlation of PTA4 of the atretic ear with speech discrimination in the cocktail party setting. Both SLA and SCS were significantly worse than in normal hearing controls, as shown in **figures 12 and 13**.

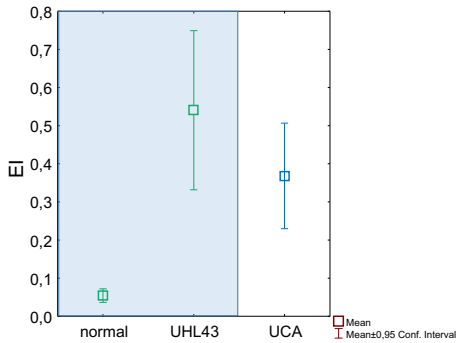

Figure 12. Results from the localization task showing that participants with ear canal atresia (UCA) performed worse than normal hearing participants. There was a tendency that UCA performed better than experimentally induced unilateral hearing loss, although this was not significant. Figure republished from [134], with permission from the journal.

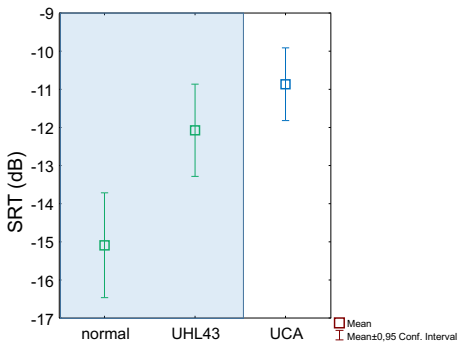

Figure 13. UCA performed significantly worse also in the speech discrimination task in a cocktail party setting. Figure republished from [134] with permission from the journal.

Study II

The acoustic radiation (AR) was tracked with probabilistic tractography (iFOD2) and anatomically constrained tractography (ACT) in scans from the human connectome project (HCP). This work was time consuming, approximately 10 hours per subject, and tractography was not possible to do in the scans of the UCA and NH of this project due to lower spatial and angular resolution. Instead, the program Tractseg was trained as described below, to extract masks of the core of the AR to be applied in our study participants.

From 105 HCP young adult brain DWI scans, masks from the tractographies of the AR were used to train the Tractseg neural network architecture (63 for training, 21 for validation and 21 test subjects). During training, the correlation of the tractographies obtained from the same subjects could be followed with increasing F1 score, as visualized in **Figure 14**. The F1-score, or the Dice coefficient (given as 0-1 where 1 is perfect overlap), is calculated as described in **Figure 14** below. The training reached a steady state, and the highest F1-score reached was 0.73.

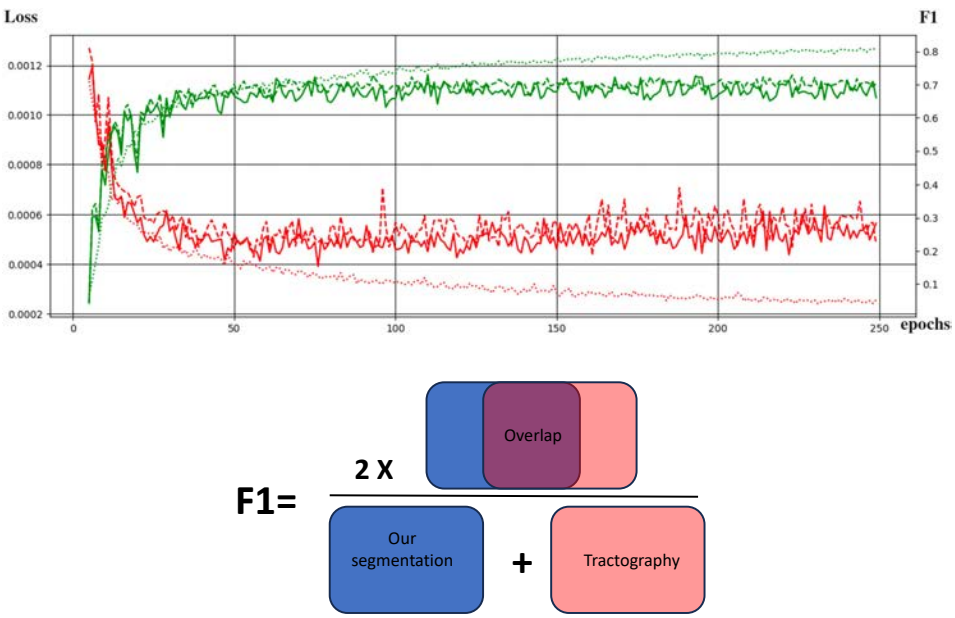

Figure 14. Uppermost, the green curve shows the increasing F1 score during training of the network in Tractseg. Below, the formula to calculate the F1, or Dice-score is shown (originally F1= (2x area of overlap (prediction and ground truth)/Total area (prediction + ground truth)). A steady state seemed to be reached at F1 around 0.70. Figure uppermost from[135], Creative Commons Attributuin Licence (CC BY). Description of Dice score modified from[136].

The trained neural network was used to segment masks in the clinical scans individually, based on DWI data. The tracking was successful in in 53 out of 68 cases (78%). Results were comparable in the UCA group and controls (24 vs. 29 tracking success). In 14 cases the method gave fragmented tracts, and the fragments could often be used as seeds to track the missing parts.

The FA values measured at several levels along the acoustic radiation, was compared with a student's paired t-test between UCA and NH right side and left side separately. There was no significant difference of the FA value on either side. **Figure 15** shows the results for each group along the acoustic radiation.

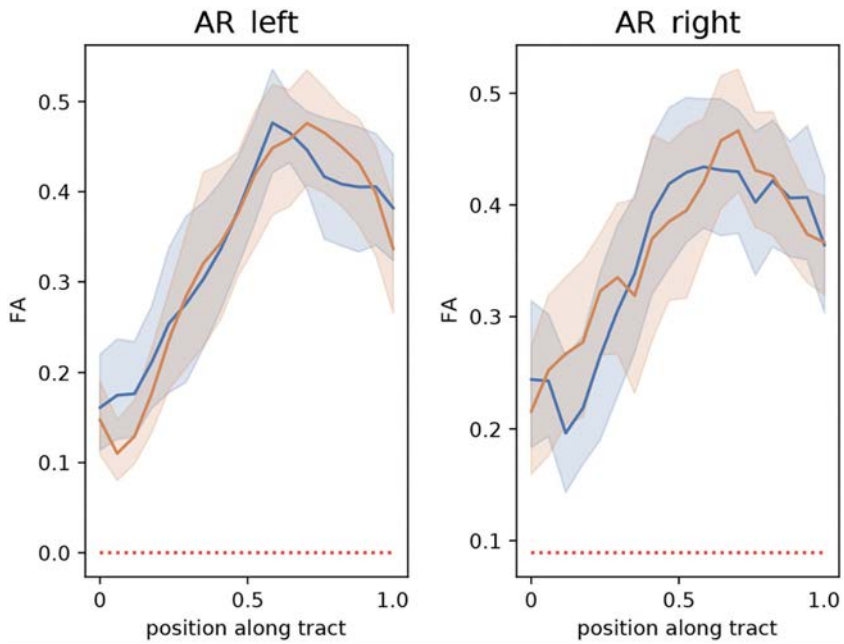

Figure 15. Results from measurements of fractional anisotropy (FA) of the acoustic radiation in participants with unilateral atresia (UCA) in blue and normal hearing controls in orange. The shaded colors are representing 95% confidence interval. The FA varies along the tract from the medial geniculate nucleus (position 0) and the Heschl's gyrus (position 1) and the curves follow each other. Figure reprinted from [135], Creative Commons Attributions License (CC BY).

Study III

Study III compared cortical thickness and volume measurement in 17 participants with UCA and 17 age- and gender matched controls. The main finding was an asymmetry in thickness in the UCA group (right thicker than left side, Bonferroni corrected p= 0.0012), which was not present in NH, shown in **figure 16**. The pattern was not reversed in left sided atresia, compared to right sided atresia.

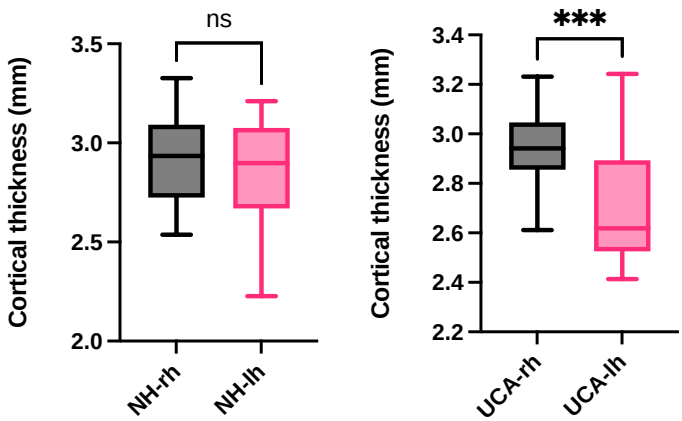

Figure 16. Cortical thickness of the Heschl's gyrus is symmetric in normal hearing controls (NH), while it is asymmetric right>left in congenital unilateral ear canal atresia (UCA).

It must also be mentioned that there was no correlation with PTA_4 with the left side HG thickness, which was found in another study [22] studying bilateral sensorineural hearing loss.

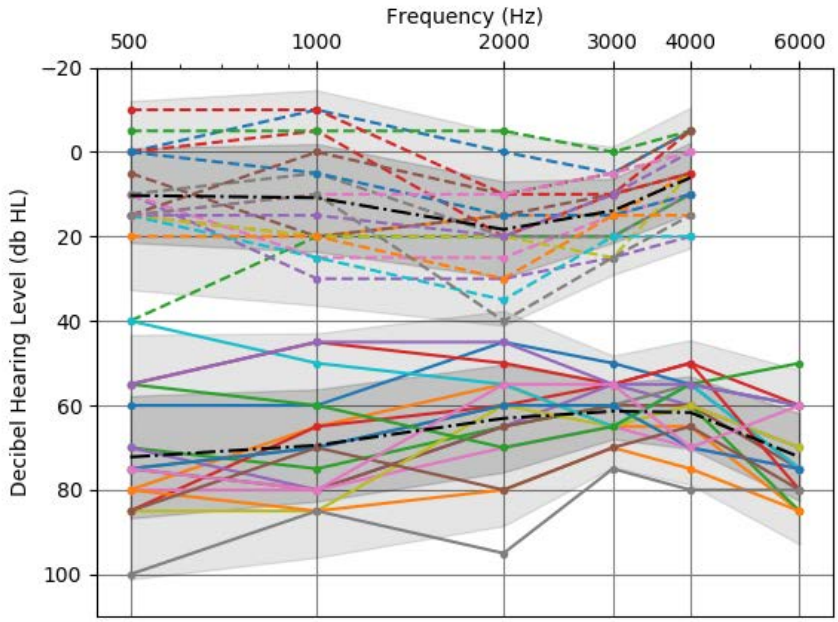

Figure 17. Hearing thresholds of the atretic ears of the participants included in **study II** and **III.** Solid lines represent air conduction and dotted lines bone conduction.

Study IV

Seven rat brains from rats with surgically constructed UCA and four controls were possible to use for this study and brains from four control rats. Unfortunately, due to failed surgery in the first cohort of rats that were operated, the ear canal was not fully closed, and the cohort needed to be discarded. An ethical permission was obtained to add more rats to the study, and the next cohort was successfully operated by a single ear surgeon co-author Engmér Berglin, and all had a fully closed ear canal. Controls were kept from the previous cohort. This led to the randomization not being perfect, and the brains had been in formaldehyde solution for 5 months in the control group, and for 1 month in the UCA group before scanning *ex vivo*.

A correction for multiple comparisons was calculated, and p-values less than Bonferroni p=0.05/4=0.0125 was considered significant (adjusted for four categories of comparisons: whole brain FA, filtered ROI-based FA, acoustic radiation and cochlear nucleus -inferior colliculus tract). P-values are given as

corrected in the text (uncorrected p*4 in significant cases) if nonsignificant they are given as p>0.05.

Table II. Results of two separate calculations of fractional anisotropy (FA) using probabilistic tractography, for the two groups of rats: rat model of unilateral ear canal atresia (UCA) and control rats (c). Results from whole brain tractography, and from tracts between defined auditory regions of interest. The FA was higher in the UCA group using both methods.

	FA, c Whole brain	**FA, UCA Whole brain**	**FA, c Filtered ROI-based**	**FA, UCA Filtered ROI-based**
mean	0.29	0.32	0.32	0.37
SD	0.008	0.007	0.013	0.009
range	0.02	0.02	0.03	0.02

There was a significant difference of the FA values using both methods of calculation, whole brain with FSL software (Mann-Whitney test, corrected p=0.0244), and between auditory ROIs from filtered tractograms (Mann-Whitney test, corrected p=0.0244), as described in **table II**.

The acoustic radiation (AR) was compared with the filtered tracts between ROIs medial geniculate body and the primary auditory cortex, UCA rats had higher FA on the right side (Mann-Whitney test, corrected p=0.0244) but not on the left side (Mann Whitney-test, corrected p>0.05). There was a difference within the UCA group found where the left side had lower FA than the right (normal distribution of data, student's paired t-test corrected p=0.0112) as shown in **figure 17.** In controls, there was no difference between the right and left side of the AR (Wilcoxon matched pairs test, corrected p>0.05).

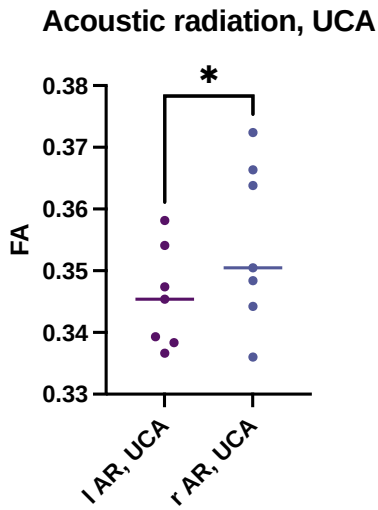

Figure 18. There was a difference between the left and the right side of the acoustic radiation in rats with UCA, where the AR ipsilateral to the ear of hearing loss had lower FA.

Based on the research by[100] where stronger projections between the cochlear nucleus (CN) to the inferior colliculus (IC) on the hearing side was found, we tested within group comparison CN-IC left versus right side, and there was a significant difference left<right (paired t-test, corrected p=0.02), shown in **figure 19**. In control rats there was no asymmetry in this tract (left CN-IC vs. right CN-IC, Wilcoxon matched pairs, corrected p>0.05).

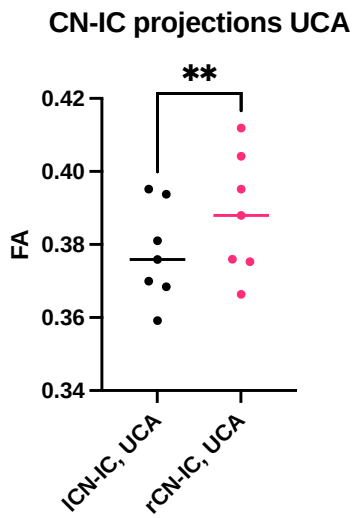

Figure 19. A difference in FA was observed in the tracts left cochlear nucleus-left inferior colliculus (lCN-IC) which had lower FA values compared to the right side (rCN-IC) in rats with left sided ear canal atresia (UCA).

5 Discussion

The aim of this research project was to explore possible differences in structure and function of the central auditory pathways in patients with unilateral ear canal atresia (UCA). Infants with UCA are offered BCD on a soft band, and sometimes the question is brought up what would happen if the child does not use it: Do the central auditory pathways "shut the ear down", is any damage possible to visualize? From the four studies of the current thesis and knowledge from previous research, this discussion attempts to summarize the findings.

The functional deficits in measurements of auditory perception in complex listening situations in **study I** show that adults who did not use any hearing aid in early life still have problems with directional hearing and discrimination of spoken language in noisy environments. There was a finding that the pure tone average of the atretic ear was correlated to performance in a sound localization task, suggesting that even a little audible sound reaching the impaired ear gives important cues to localize. These results are supported by findings in two other studies where presentation level of stimuli was assessed in localization in unilateral conductive hearing loss [41,42]. Speech discrimination in competing speech however remains unclear why some participants scored higher. All participants spoke fluent Swedish.

In **study II**, one of the aims was to find possible changes in the acoustic radiation in UCA as compared to controls. A method was developed through probabilistic tractography and CSD on high quality DWI data which was used to train an algorithm to segment the core of the AR with a few quick steps in our scans based on diffusion data. In our material, data from a clinical DWI scanning session was collected, which means that the angular and spatial resolution is low, and it can be more challenging to run tractography successfully. In an experimental study on rats, disrupted tonotopy was found at the primary auditory area in early unilateral ligation of the ear canal[19]. The core of the acoustic radiation is thought to have a tonotopic organization because it connects the ventral part of the MG with the HG as shown in animal studies [48–50,137]. It is challenging to study the acoustic radiation because of a lot of adjacent crossing larger white matter bundles, although the results from the attempt presented looked promising upon visual inspection and comparison with probabilistic earlier described masks. Success rate was comparable in UCA and NH, 24/34 vs 29/34 complete tracking rate and using the mask as a seed to run tractography quicker was

possible in almost all cases with incomplete definition of the AR. The graphs presented in **figure 15** indicate a difference in FA along the AR tract both in UCA and controls, which is interesting. This is to our knowledge not presented before, and could be due to adjacent crossing fibers, or different orientation or density of fibers at different parts of the AR. The confidence interval seemed even larger on the right side than the left side, and together with the fitting with the mask that was less successful on the right than the left side, this may reflect, and add information to the asymmetry of this tract previously described[52]. There was also a larger variability between subjects in FA of the AR, as compared to other larger white matter tracts. There was no difference between UCA and controls in FA of the right or the left AR. FA is a measurement that can give indication of nerve damage or atrophy in clinical settings in other diseases[83,84]. If the acoustic radiation is not preserved in UCA, the changes were not possible to detect with this outcome variable in clinical scans. To compare, **study IV** with post-mortem scans in rat brains, we found higher FA in the right AR in rats with UCA than in controls, and a within UCA group difference, where the right side had higher FA values than the left. We did not analyze within group measurements (left AR vs. right AR) in **study II**. However, this may indicate that a longer DWI scanning time in human participants and higher angular resolution could reveal changes. In rats, it was not possible to visually inspect the origin if the FA measurements, since the tractography with filtering was made on whole brains, and FA measurements were extracted between ROIs and presented as a mean value.

The grey matter of the primary auditory cortex (PAC) of the Heschl's gyrus (HG) was measured in **study III**, in the same 17 UCA participants and 17 NH controls as in **study II**. Volume measurements were normalized to total intracranial volume, and mean thickness of the HG were used for comparisons. One of the most challenging tasks in this study was how to compare data. In normal hearing participants, the left PAC not only represents the right ear stronger than the left, but also has a relatively specialized function in processing more language like sounds (rate related changes) and the right PAC represents the left ear and is more specialized in spectral related changes in auditory stimuli[62]. In UCA it is not known if this specialization still exists, although in right-sided congenital deafness there is commonly a dominance of the language function of the left hemisphere, as in NH subjects [74]. The decision was to compare left and right HG within the groups, and also in subgroups with left UCA and right UCA, instead of grouping with "ipsilateral, or contralateral HG to ear of hearing loss". Interestingly,

the pattern of volume and thickness of the left and right HG was not reversed in left-sided UCA vs right-sided UCA. We found an asymmetry in cortical grey matter thickness, left< right in all of the UCA group (left- and right-sided UCA) which is not present in NH. It is not clear whether this reflects a functional deficit in language processing of the left side, however the left HG thickness was not correlated to the level of hearing loss in the atretic ear as reported in bilateral acquired hearing loss [22]. There was no difference in total thickness of the left + right HG, nor any difference in total volume compared to NH.

Study IV examined white matter tracts in post-mortem rat brains from rats with surgically constructed UCA (before onset of hearing, n=7) and in controls (n=4). These scans minimized off distortion-artifacts through removal of external ears and scanning in a fatty solution, and magnetic fields were stronger (9.4 T compared to 3 T in human scans), scanning time was several hours/rat brain.

Unfortunately, time from euthanasia to scan differed on a group level, where the control rat brains waited approximately 5 months to scanning in formaldehyde, and the UCA group only 1 month. There is a study on scanning parameters changing over time in human brain specimens preserved in formaldehyde were studied for 41 days, where FA was not different over time[138], however this systematic error due to lack of randomization must be discussed.

Therefore, findings within groups of differences in laterality pattern are more of interest:

The FA in the acoustic radiation between the medial geniculate body (MG) and the primary auditory cortex (PAC, or shortened AC in the studies on rats) is also discussed above, along the findings in **study II**. The left side AR had lower FA than the right side, which means the ipsilateral cortex relative to the ear of hearing loss had lower FA. The higher FA values of the right AR could be due to less crossing tracts from the primary auditory area to other nearby cortical structures, or that rats also have a specialized function of the left vs. right primary auditory areas, as in human where language is a commonly left-sided dominant function, and that the asymmetry reflects a functional deficit in rats with UCA.

We also found that the left side (side of hearing loss) had lower FA between the cochlear nucleus (CN) and inferior colliculus (IC) than the right side in UCA, whereas there was no difference between left and right in controls. This finding

aligns with results presented in ferrets with early onset unilateral conductive hearing loss, where projections between these structures were negatively affected on the side of hearing loss[100].

6 Conclusions

There are no signs of atrophy of the primary auditory cortex (PAC) in adults with UCA, however an asymmetry in thickness of the structure was observed (left<right). It was possible to segment the core of the AR, the white matter tract between the MG and the PAC, in a few seconds in most cases of clinical scans, using a pretrained neural network. The AR was not different when measuring FA (diffusivity measurement) in human participants with UCA compared to controls, but an asymmetry in FA of the AR was found in rat brains from rats with UCA which is not present in controls. Subcortical white matter tracts were also affected in rats with UCA. Sound localization and speech in competing speech measurements in adults growing up with UCA and no hearing aid was significantly worse than NH. The sound localization ability was dependent on hearing level of the atretic ear, but language discrimination was not.

7 Points of perspective

From the results of the studies included in this thesis, there are functional deficits in auditory perception in UCA that can be measured with speech perception tasks and sound localization tasks. Imaging of this condition in our studies did not reveal changes that can be used in individual cases. Measurements of white matter integrity still have a lot of methodological challenges, and the method description included in the thesis on studying of the acoustic radiation can still be improved with better data acquisition and processing. Patients with UCA and especially parents of young children with the condition, where functional testing is limited, are asking for a better prognostic tool to determine if the individual child would benefit from a hearing aid solution. Although from the results of **study I**, where we can predict that worse hearing as measured by pure tone average of the audiogram of the atretic ear is associated with worse sound localization ability, the question remains how to predict ability to discriminate spoken language in noisy conditions. It must also be said that a few participants with severe hearing loss of the atretic ear, actively said that they did not have any problems in their daily life related to the hearing loss. One participant even worked as a communicator and spoke three different languages and used no hearing aids.

The results reported from other studies concerning aided thresholds, localization ability and speech perception with active bone conduction implant devices in this patient group are very good, and future studies of very early implantation in toddlers are interesting to follow.

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My main supervisor for the later years of the project, **Cecilia Engmér-Berglin**, who has always been there for me, and encouraged me when I wanted to give up. You truly are a treasure, not only as a team leader for research but also for personal guidance and for our research travels. Thank you also for your generosity. I can not thank you enough!

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