8-12-2019 10:00 AM

Differential Thickening and Thinning of Auditory Cortex in Deaf Cats Revealed with Ultra-High-Field MRI

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Graduate Program in Neuroscience
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

Abstract

In the absence of hearing, the brain must adapt and repurpose the former auditory cortex. In this study we scanned normal hearing (n=29) and deaf (n=26) cats to identify cortical areas of differing thickness using the auditory regions from a 3D cortical atlas. Compared to hearing controls, differential thickening and thinning was observed in specific regions of the deaf auditory cortex. More dorsal auditory regions tended to be bilaterally thicker in the deaf group, while more ventral regions in the left hemisphere were thinner. The location and nature of these changes creates a gradient along the dorsoventral axis wherein dorsal auditory cortical fields are thickened while more ventral fields are thinner in deaf animals compared to hearing controls. Whether this reflects the spatial separation between the functionally distinct “where” and "what" pathways, or a more general property of distance along the dorsoventral axis of cortex remains to be examined.

Keywords

Cortical thickness, cat, deaf, structural MRI, auditory cortex, plasticity

Acknowledgments

I would first like to thank my supervisor, Dr. Stephen Lomber, for his continued generosity, support, and patience with me. You gave me the freedom to choose my own project (and let me change projects completely after a year) and all resources I needed to complete it. You were indispensable in the creation of this document.

My advisory committee has been instrumental in guiding me and my project to completion. I thank Brain Corneil, Brian Allman, Mark Daley and Ali Kahn for their time and expertise.

A significant number of structural scans for this thesis were obtained in conjunction with another lab on campus. To Blake Butler, thank you for sharing some of your 7T scan time with me for my project. Thank you for also putting your time into editing this document.

Summary for Lay Audience

The brain is a highly complex organ, primarily composed of grey matter and white matter. Grey matter consists of neurons which process information, as opposed to white matter which consists of axons that carry information between these neurons throughout the brain. Therefore, neurons communicate with one another through a dense network of white matter. However, this grey-white matter network is subject to adaptations in response to changing environmental conditions- a phenomenon known as plasticity. While undergoing plasticity the relative amounts of the different components of grey matter can change. These compositional changes affect the space requirements for the grey matter and can be observed as the change in thickness of cortical grey matter locally or throughout the brain. In human studies researchers have found regional differences in grey matter thickness in certain disease states as well as thickness differences in experimental groups with large amounts of experience in specific tasks, such as playing music or video games. Grey matter thickness can also be used as an indicator of Alzheimer's disease. Changes in human auditory cortex thickness have been found in different deaf groups in the past with mixed results. To take a different approach to the question of human thickness after deafness, this work used the deaf cat as an animal model of human deafness. Cats have very similar auditory cortices to humans and have been used for many years in auditory and visual neuroscience. In this work structural MRI scans of mature hearing and deaf cats were used to find any regional differences in cortical grey matter thickness of auditory cortex. It was discovered that five auditory cortex regions were thicker on both sides of the brains of the deaf cats relative to the hearing controls, and two regions on the left side were thinner. A pattern of thickness change emerged between the two groups, such that physically higher-up auditory regions in the brain were thicker in the deaf groups, and lower regions were thinner. To discover the nature of these changes further work will need to be conducted.

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Chapter 1

1 Introduction

When a sound reaches the cochlea, the signal encoding its features passes through a series of stations in the brainstem, midbrain and auditory thalamus and into auditory cortex, where it is analyzed and turned into a percept. Auditory cortex is designed specifically to discern small differences in pitch, tone, tempo and many other aspects of sound that we may not consciously aware of. With so many characteristics of sound to dissociate, the auditory cortex has developed subregions that are specialized to analyze particular components of the sound, and subregions that combine acoustic stimuli with other senses. These multisensory areas make up a large percentage of our cerebrums and the cerebral cortex of many animals. The constant influx of information allows for an always-adapting auditory cortex, with mature experimental animals showing enhanced areal representation of sounds pertinent to their training (Noreña, Gourévitch, Aizawa, & Eggermont, 2006; Witte & Kipke, 2005). But what happens when all auditory input is removed from this system? It has been shown previously that there is a degree of functional plasticity (Lomber, Meredith, & Kral, 2010) as well as possible connectional changes in the deaf cat brain (Butler & Lomber, 2013; Chabot, Butler, & Lomber, 2015; Kok, Chabot, & Lomber, 2014), but what happens to the thickness of the cortical sheet? Using structural Magnetic Resonance Imaging (MRI) in an ultra-high-field 7-Tesla scanner, the thickness of auditory cortex was measured in neonatally-deafened cats as well as normative controls to assess what happens to the thickness of the 14 cat auditory cortical regions.

1.1 Neurons and Synapses

The mammalian brain is composed, in simple terms, of grey matter (GM) and white matter (WM). WM contains mainly axons that carry information and supporting cells to ensure the proper functioning and health of these axons, neurons. GM, on the other hand, has within it the cells that send and receive these axons, neurons. Neurons are the computational units of the central nervous system (CNS). They work by taking in anywhere from one to thousands of inputs and integrate them together to determine if they themselves will send out a pulse of information, or action potential. Surrounding the neurons in the GM are numerous supporting cells. The neurons possess

appendages called dendrites that the neurons use to receive incoming information. Axons from other regions in the brain (as well as local ones) branch many times, and each branch ends in a synaptic bouton that will interact with a spiny process on a nearby dendrite. This connection is called the synapse and each one can be temporary or relatively permanent in the adult brain. Changing the number and type of synapses that are active on any given neuron can change the way that that neuron responds to specific stimuli. In contrast to synapses, neurons themselves are more permanent fixtures in the brain, and will typically only change in size slightly when they are not used instead of undergoing apoptosis and changing the total number of neurons in a given area after total sensory deprivation (Cragg, 1975; Saada, Niparko, & Ryugo, 1996). Even though these changes are microscopic in scale, the end result of large populations of neurons changing their inputs can be visualized at the scale of MR images.

1.2 Structural Magnetic Resonance Imaging

MRI uses large magnets in conjunction with radiofrequency coils to generate a 3D image of the inside of objects. To do this, a strong magnetic field is first created inside the bore of the scanner. This field aligns protons inside the body being tested with the magnetic field. These protons are then perturbed by a radiofrequency pulse. When the radio wave is stopped, the protons return to their aligned state and the speed at which they realign is measured using a receive radiofrequency coil. This longitudinal relaxation time (T1) differs by tissue composition, and tissue types can be distinguished by the intensity of voxels on a T1-weighted image (Faster realignment creates a brighter spot on the voxel). Because of the large difference in the composition of GM and WM, there is a strong contrast between the two tissue types, with GM appearing as a lower intensity than WM (Figure 1.1). An added benefit of using T1-weighted imaging is that the cerebrospinal fluid (CSF), which surrounds the GM of the cerebrum, is very low-intensity and does not interfere with thickness measurements. To obtain the images used in this study, a modified version of the Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE), called MP2RAGE, was used (Marques et al., 2010). This is a sequence that can generate a T1WI very quickly and with a good signal-to-noise ratio (SNR). To generate full brain images in the cat for the current work, 96 slices at 0.5mm isotropic resolution were captured over the course of ~9 minutes per subject.

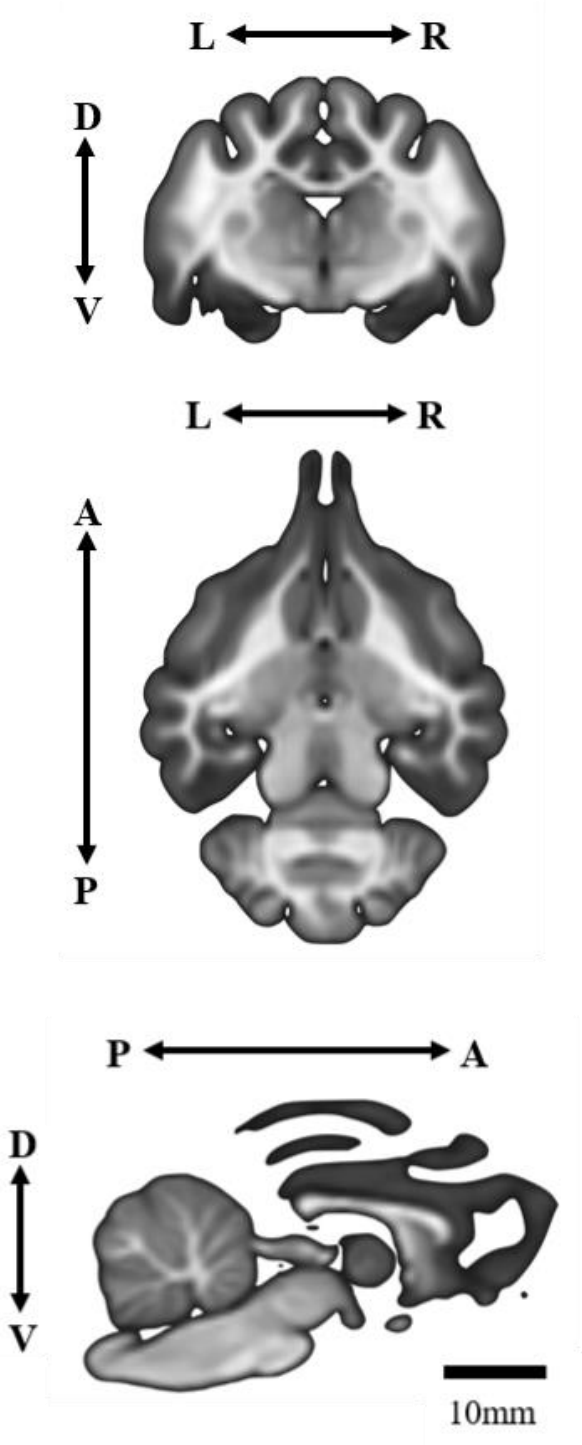

Figure 1.1 Example sections of the template used in the present study. Coronal (top), horizontal (middle) and sagittal (bottom) views are all shown. Grey matter (GM) appears darker and white matter (WM) appears lighter in this T1-weighted image.

1.2.1 Grey Matter Thickness as Determined by MRI

In this study we examined GM cortical thickness (CT) to find changes between hearing and deaf brains. It is important then to know what determines CT, both in a real, physical way as well as what is seen in an MR image. Physical GM thickness is determined by neuronal density, glia (the support cells of neurons), and the axons and dendrites coursing around these cells. Due to the fact that underused neurons tend to shrink instead of undergoing apoptosis (Cragg, 1975; Saada et al., 1996) and glia are always required to be present to care for and clean up after neurons, the most important factors determining GM thickness are neuronal cell size and synaptic density (Bavelier & Neville, 2002; Shefer, 1973). Luckily, the unmyelinated axons and dendrites surrounding neurons in GM lower T1 relaxation times of the surrounding tissue, enhancing the GM/WM contrast. As the synaptic density increases in any given region, the T1WI intensity decreases, even in the tissue around the GM/WM border. This means that in an MR image it is possible for automated software to shift the GM/WM border more or less than it has really moved, but an increase in image GM thickness should correlate to an increase in real-world GM thickness. This is why it is very important to always have a control group when performing studies on CT as determined by MRI, as absolute measurements could be inaccurate but relative changes are more concrete.

1.3 Human Cortical Thickness

Being non-invasive, MRI has been used in many studies of human cortical thickness in recent years. This work covers many topics in relation to cortical thickness, including intelligence quotient (Narr et al., 2007), meditation experience (Lazar et al., 2005), age and sex differences (Lemaitre et al., 2012; Luders et al., 2006), video game hours played per week (Kuhn et al., 2014), and more. There have also been studies looking at the auditory system and the brain. Thickness differences have been found between the brains of subjects of amusia (a disorder in the perception of pitch and/or musical recognition) and healthy controls (Hyde et al., 2007) as well as between musicians with and without absolute pitch and healthy controls (Bermudez, Lerch, Evans, & Zatorre, 2009). These studies from the Zatorre group show that there are observable structural differences that can be found given an underlying auditory condition, albeit one that still maintains hearing. Deafness has also been analyzed from this group, with interesting results (Penhune, Cismaru, Dorsaint-Pierre, Petitto, & Zatorre, 2003). While there was limited change to the auditory

cortex grey matter in thickness or volume, white matter volume was found to have changed, specifically around Heschl's gyrus. Interestingly, left/right Heschl's gyrus asymmetries were preserved in the deaf, suggesting a strong genetic driving factor behind these features of the brain. This could also imply that any new function of previously auditory cortex would rely on connections laid out before any hearing experience could be achieved. Thickness can change due to experience, but that effect is constrained by the underlying architecture of the brain.

1.4 Animal Model of Human Deafness

The domesticated cat (*Felis Catus*) is an important model animal in science, as it helps bridge the gap between rodent and primate research. Cats can reproduce rapidly, with a gestation period of two months and litters of between 2 and 7 kittens on average. Cats mature in 6 months, when they can then be trained to perform simple or complex behaviours, such as sound localization, visual motion detection, or hindlimb obstacle avoidance (Jones, Ruhland, Gai, & Yin, 2014; Lomber & Malhotra, 2008; Lomber et al., 2010; Malhotra & Lomber, 2007; Malhotra, Stecker, Middlebrooks, & Lomber, 2008; Wong & Lomber, 2019). After training these subjects, electrophysiological recording techniques and reversible or permanent deactivation approaches can be used to glean the function of specific cortical regions. Electrophysiological recordings obtained from cats are very useful as their auditory cortex is much more similar to ours than that of rodents and so we can test more complex theories about the human brain. We can also access much more of their auditory cortex than that of humans or non-human primates since a large portion of cat auditory cortex is on the outer surface of the brain and not hidden in fissures or sulci (Figure 1.2). For this reason and others, the cat has been used in auditory and visual neuroscience for many years as a model between rodents and primates. Much work has gone into electrophysiological and cytoarchitectonic studies to help classify all the subregions of auditory cortex and how neurons in these subregions react to many different stimuli (Carrasco & Lomber, 2009, 2011; Jenkins & Merzenich, 1984; Merzenich, Knight, & Roth, 1975; Middlebrooks, Dykes, & Merzenich, 1980).

Along with its characteristics in the normative state, many studies have examined the functional consequences of visual or auditory deprivation on the cat brain (Butler & Lomber, 2013; Cragg, 1975; Land et al., 2016; Lomber, 2017; Meredith & Lomber, 2011). Anatomically, many studies have used retrograde (Butler, Chabot, & Lomber, 2016; Butler & Lomber, 2013; Chabot et al., 2015; Kok et al., 2014; Meredith, Clemo, Corley, Chabot, & Lomber, 2016; Wong, Chabot, Kok,

& Lomber, 2015) tracers to determine changes in connectivity and synaptic density among regions after deafness. There have also been behavioural studies (Lomber et al., 2010) showing that certain regions in the deaf auditory cortex contribute to visual tasks that they did not previously. This kind of functional plasticity has been shown to be linked to specific regions of auditory cortex (Lomber et al., 2010). In this work specific regions of deaf cat auditory cortex are temporarily deactivated during a visual task. During the deactivation period of individual auditory cortex regions, the deaf animals lose their visual advantages. The underlying nature of the functional plasticity is still unknown but considering the lack of consistency in reorganization seen in retrograde tracer studies (Butler et al., 2016; Kok et al., 2014), it would appear that these changes are mediated locally, in the form of dendritic branching, reweighting of inputs and changing the total number of synapses.

1.4.1 Cat Auditory Cortex

Based on past cytoarchitecture staining and functional studies, the atlas used in the current study has auditory cortex divided into 14 distinct regions. These regions have been delineated based on function and anatomical connections (Lee & Winer, 2008a, 2008b, 2008c). One such grouping is the distinction between the "what" and "where" auditory pathways (Figure 1.2), along with the two primary regions, anterior auditory field (AAF) and primary auditory cortex (A1) (Lee & Winer, 2011). These pathways process the spectral features of an auditory percept and its spatial location, respectively. The "what" pathway consists of the ventral division of the posterior auditory field (vPAF), second auditory cortex (A2), ventral auditory field (VAF), insular cortex (In), temporal auditory cortex (T), and the ventral division of posterior ectosylvian cortex (vPE). The "where" pathway contains the remaining auditory cortex regions; posterior auditory field (PAF), the dorsal zone of auditory cortex (DZ), the auditory field of the anterior ectosylvian sulcus (fAES), and the dorsal, posterior, and intermediate divisions of the posterior ectosylvian cortex (dPE, pPE, iPE). There are other possible functional groupings of auditory cortex. For example, a subset of these 14 areas (AAF, A1, PAF, VAF, and vPAF) are organized tonotopically (Hall & Lomber, 2015; Imig & Reale, 1980; Lee, Imaizumi, Schreiner, & Winer, 2004). Tonotopy is the spatial arrangement of neurons wherein neurons that are closer together have similar frequencies of sound that they are most responsive to.

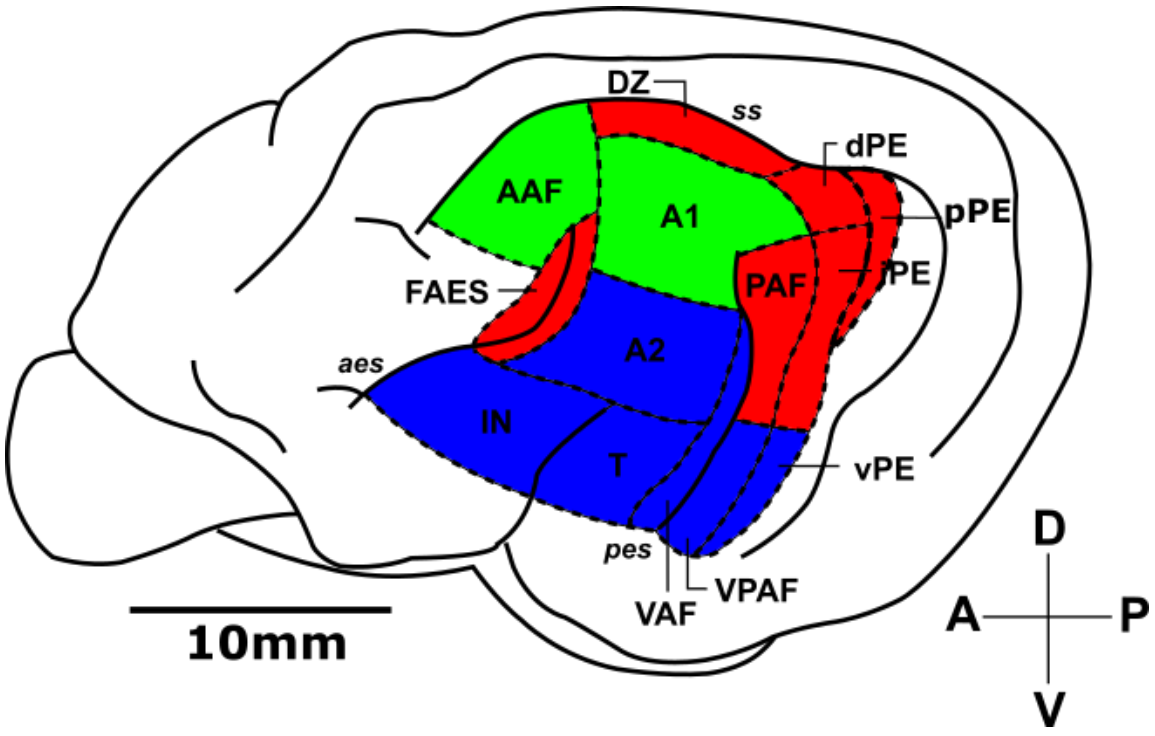

Figure 1.2 Visual representation of core (green) auditory areas as well as the what (blue) and where (red) regions of the cat auditory cortex. Adapted from (Lee & Winer, 2011; Lomber & Malhotra, 2008).

1.4.2 CATLAS

Until recently, a three-dimensional cortical atlas of the adult cat has not existed. To remedy this, Stolzberg and colleagues (Stolzberg, Wong, Butler, & Lomber, 2017) used past histological and electrophysiological atlases and high-resolution MRI scans of mature cats to design and model an atlas which they called the Catlas. This atlas contains 71 bilateral cortical regions and 9 "other" regions which include the cerebellum and midbrain/brainstem grey matter structures. Lines defining areal borders were created using lines of best agreement between multiple atlases and drawn onto a three-dimensional average brain. The files provided with the Catlas also contain the average image of 8 mature animal scans that is in alignment with the atlas, allowing for easy normalization and use of data in other studies. In the current work only the 14 bilateral auditory areas are used, but future work could examine visual, somatosensory, and "other" regions.

1.4.3 Cortical Thickness in Deaf Cats

A good deal of inspiration for the current work was driven by cytoarchitectonic work conducted by Berger and colleagues (Berger, Kühne, Scheper, & Kral, 2017). In this work, coronal sections from the brains of congenitally deaf cats contained A1, A2, DZ and Area 7 (a control visual area) were analyzed for cortical thickness of these regions. Compared to hearing controls, visual Area 7 was unchanged, and A1 and A2 were both found to be thinner in the deaf group. Upon further examination, it was found that cortical layers IV-VI of A1, A2, and DZ were all significantly thinner in the deaf group. The overall effect size of this study (~0.2mm) and results of this work were used to determine the number of subjects for the current work and to guide the predictions.

1.5 Goal of the Current Study

In the current study we aimed to examine the extent to which the thickness of specific subregions of auditory cortex changes between hearing and neonatally-deafened cats using ultra-high-field 7T MRI. We hypothesize that the underused auditory cortex in the deaf animals will have shrunk in thickness relative to the hearing group. Using structural T1-weighted images and the open-source software ANTs, mean thicknesses for each of the 14 bilateral auditory cortex regions were assessed and compared across the two groups. Due to the proposed effects of synaptic pruning, and based on past evidence (Berger et al., 2017), it is predicted that the auditory cortex of the deaf group will be thinner than the hearing controls. Specifically, A1 and A2 are expected to be significantly

thinner in the analysis, as DZ in Berger's work had very minimal overall thickness changes and was only found to have changed on the scale of cortical layers which can't be separated in these MR images. The work here will lay groundwork for future studies in which the nature of any changes can be examined in further detail.

Chapter 2

2 Materials and Methods

All procedures were approved by the University of Western Ontario's Animal Use subcommittee of the University Council on Animal Care and were conducted in accordance with the guidelines specified by the Canadian Council on Animal Care.

2.1 Animals and Deafening

Twenty-three mature cats (13 hearing, 10 deaf) were used for the generation of the average template and a total of 55 cats (29 hearing, 26 deaf) were used in the analysis. The deaf animals were deafened early in development using the ototoxic antibiotic neomycin (Leake, Hradek, Rebscher, & Snyder, 1991). From P1, kittens were subcutaneously administered neomycin on a daily basis until hearing thresholds were above 80dB SPL as determined by auditory brainstem response (ABR, Figure 2.1). In general, threshold shift was observed by P25-P30. Follow-up ABRs were performed at least 3 months later to confirm deafness in these animals, while hearing animals underwent one ABR to verify normal hearing levels prior to scanning.

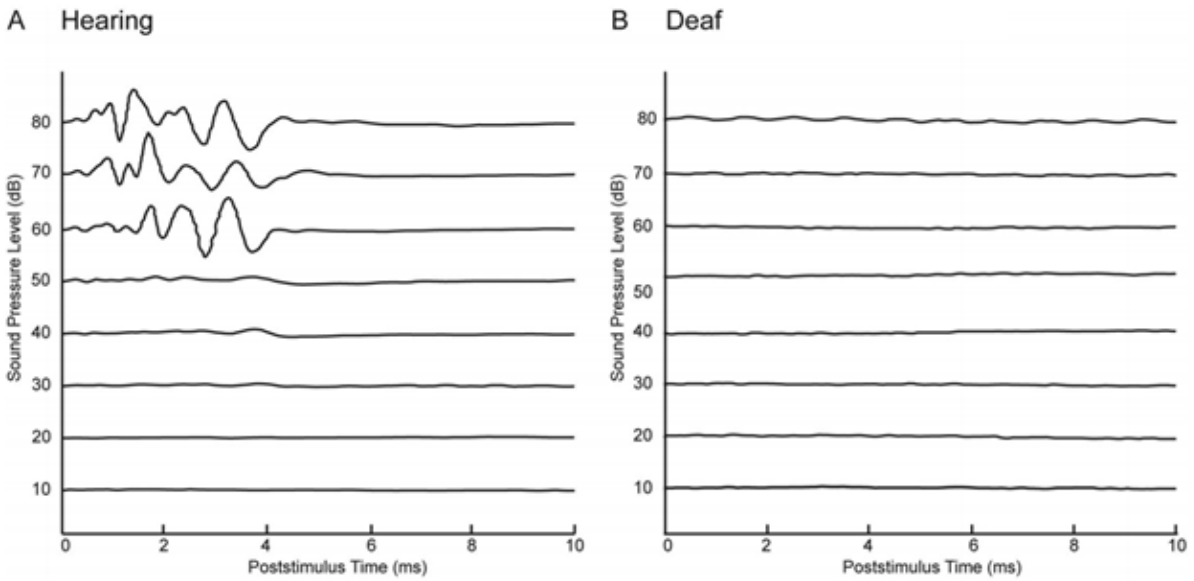

Figure 2.1 Example auditory brainstem responses (ABRs) of both groups used in this study. (A) Representative ABR of a hearing animal showing the characteristic waves with increased latency at lower stimulus levels, obtained between the ages of 4 and 6 months. (B) Representative ABR of an ototoxically-deafened animal after onset of deafness (around P30), showing no discernible waves to any click stimulus level up to 80dB. Three-month follow-up ABRs for the deaf animals were also absent of waves. Adapted from (Wong et al., 2015).

2.2 Anesthesia

Cats were anesthetized according to previously established protocols (Brown et al., 2013; Hall et al., 2014). Prior to each imaging session, cats were pre-medicated with a mixture of atropine (0.02 mg/kg s.c.) and acepromazine (0.02 mg/kg s.c.), then anesthetized ~30 min later with a solution of ketamine (4 mg/kg i.m.) and Dexdomitor (0.022 mg/kg i.m.). Upon confirmation of an absent gag reflex, each animal was intubated and an indwelling catheter was placed in the saphenous vein to facilitate intravenous delivery of fluids and anesthesia. Once prepared, the animal was placed in a sternal position within a custom-built apparatus. Anesthesia was maintained during each session with ketamine (1.2–1.8 mg/kg/h i.v.) and spontaneously inhaled isoflurane (~0.5%) with medical oxygen (~1.5 L/m). After scanning was complete, animals were recovered until sternal recumbency was achieved, at which point they are placed into individual housing until the next morning. The next morning they were returned to the colony.

2.3 Image Acquisition

Structural MRIs were taken using a 7 T Siemens Magnetom MRI scanner (68cm bore diameter) operating at a 350 mT/m/s slew rate and a custom manufactured 8/24 channel transmit/receive radio-frequency coil (Gilbert, Gati, Barker, Everling, & Menon, 2016). A high-resolution T1-weighted MP2RAGE image was acquired for each subject (repetition time (TR) = 6500ms, echo time (TE) = 3.93ms, flip angle 1 = 4°, flip angle 2 = 5°, 96 slices, 0.5 mm isotropic voxel size).

2.4 Image Preprocessing

Raw DICOM files were converted to the compressed Neuroimaging Informatics Technology Initiative (NIfTI) file format and manually reoriented into stereotaxic space using an open-source software (3D Slicer; http://www.slicer.org), placing the image origin at the stereotaxic origin. The images were then all manually skull-stripped using MRIcron (NeuroImaging Tools & Resources Collaboratory). To create the average template image, 23 of these brain-only images were combined using the *MultivariateTemplate-Construction2* function from the Advanced Normalization Tools (ANTs) software toolbox. Tissue probability priors were then created from this template for GM, WM, and cerebrospinal fluid (CSF) using *antsAtroposN4*. The GM prior was then manually separated into cortical grey matter, deep grey matter, brainstem and cerebellum. The template and its priors were then used in the *antsCorticalThickness* pipeline, modified slightly

to account for the size of the cat brain. All subjects' images then had thickness maps extracted using this pipeline. The cortical GM thickness maps were then aligned using nearest neighbour interpolation to a digital atlas of the cat brain (Stolzberg et al., 2017) and region classifications were obtained for 14 bilateral auditory regions of interest (ROIs) based on the atlas.

2.5 Advanced Normalization Tools

Diffeomorphic Registration-based Cortical Thickness (DiReCT) is the method that the ANTs cortical thickness pipeline utilizes to generate thickness maps (Das, Avants, Grossman, & Gee, 2009). DiReCT first determines the GM/WM and GM/CSF borders. It then three-dimensionally draws the GM/WM surface and grows it in the direction of the GM/CSF border (Figure 2.2). Once the GM/CSF border is reached, all voxels along the growth line are filled with an intensity equal to the measured thickness of the tissue in millimeters. In this way, a thickness map is generated in which voxel intensity is related to local thickness of the cortical sheet.

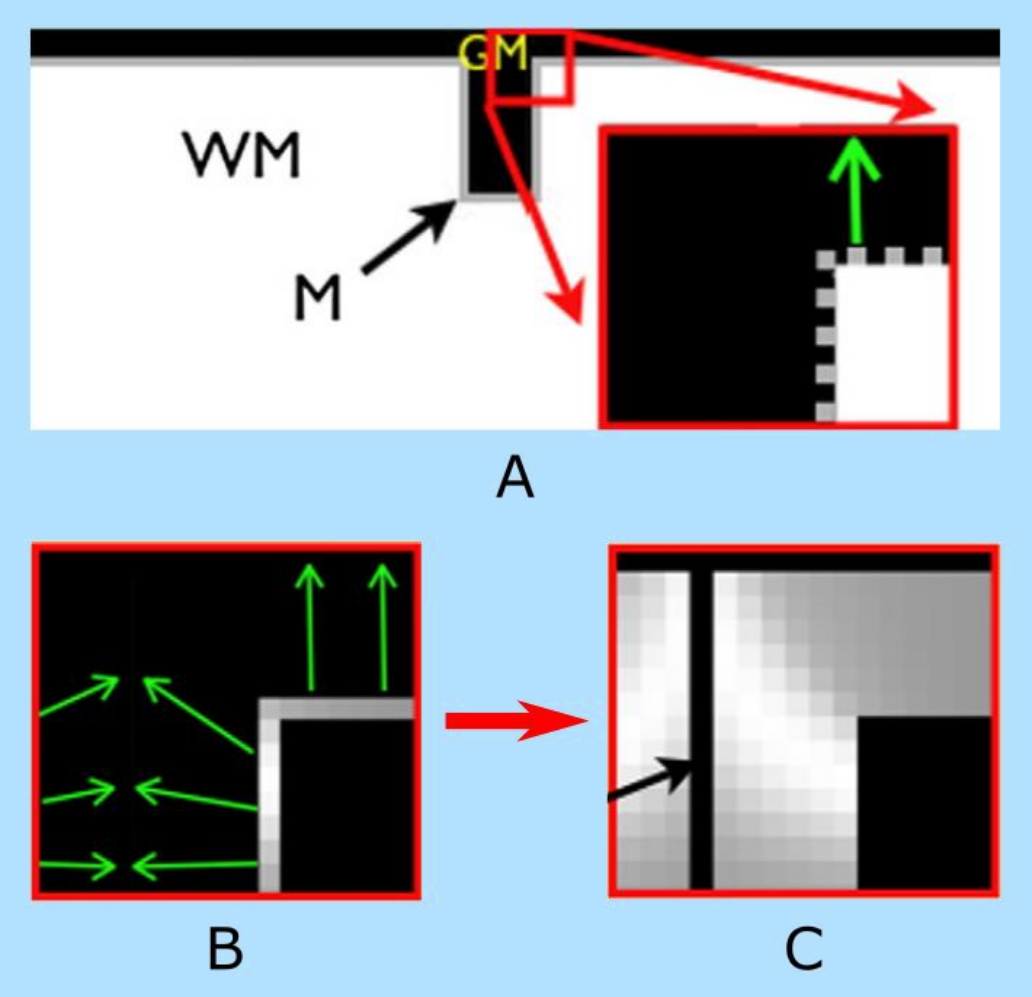

Figure 2.2 Visualization of the methodology used to generate thickness maps in the DiReCT algorithm. Grey matter (GM) and white matter (WM) are denoted in (A) as black and white, respectively, with the modeled GM/WM interface in grey and marked with an M. Green lines indicate the direction of growth in (A) and (B), with (C) showing the end product. The black arrow in C indicates that the software can identify sulci and will not overestimate thickness in folds. The final image intensity values in (C) are equivalent to the measured thickness of that column of tissue in mm. Figure adapted from (Das, Avants, Grossman, & Gee, 2009).

2.6 Raw Thickness Values

After alignment to a common space, there were regions in different subjects in which a voxel did not contain a thickness value due to differences in overall brain size and warping accuracy. To ensure that mean cortical thicknesses would not be affected by any of these voxels, all subjects' images were superimposed upon one another in a common 3D space with the cortical atlas. Voxels without thickness values in any given subject were removed from all images for analysis. This process ensures consistency in the number of data points used. Mean values were then obtained for each auditory ROI in the Catlas.

2.7 Processing of Thickness Measurements

The effect of age was regressed out on a per-region basis to account for differences in growth of any given region over time, followed by normalization of the mean cortical thickness of each subject versus the population average (Figure 2.3). The mean of these thickness values was then obtained for both groups in each region, and the significance threshold was adjusted using MATLAB R2015b's (Mathworks) *mafdr* false discovery rate function with a q value of 0.05 using the Benjamini and Hochberg (Benjamini & Hochberg, 1995) method due to the small sample size of 28 regions.

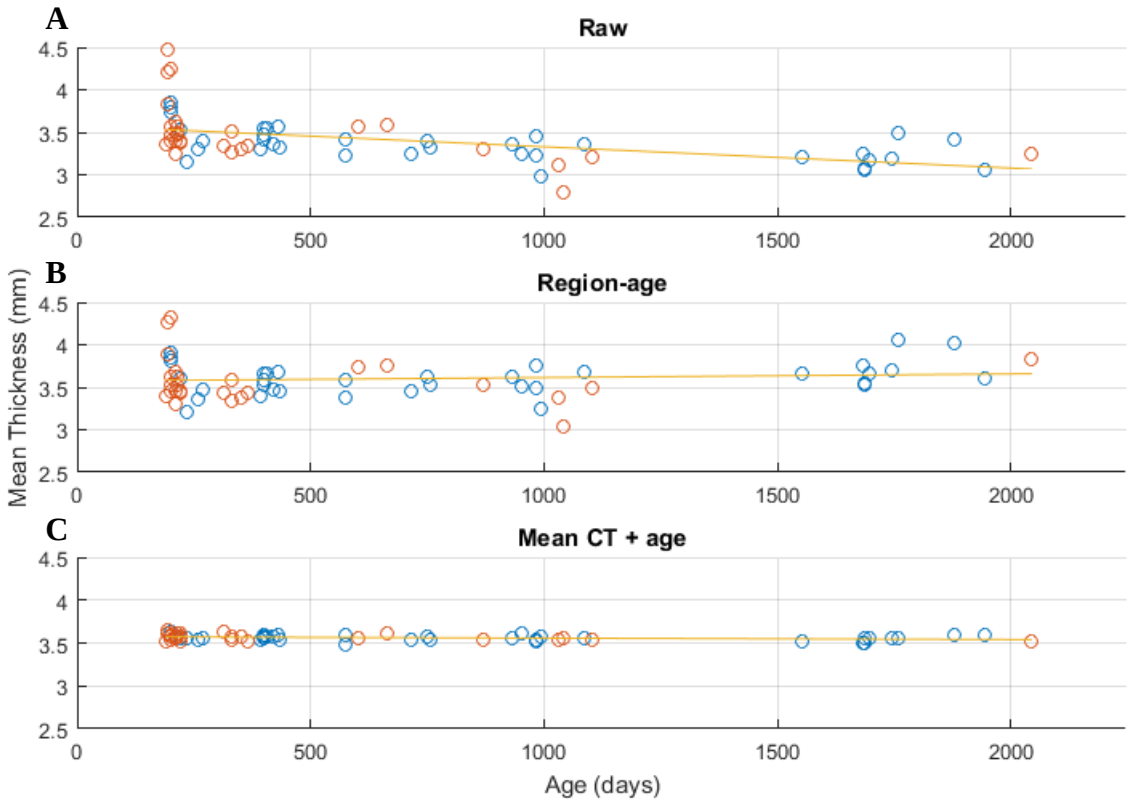

Figure 2.3 Normalization of mean cortical thickness (CT) values based on age and mean CT of the hearing (blue) and deaf (red) groups. (A) Raw mean CT values for each subject in both groups. (B) Mean CT values after normalization based on the region-specific changes over time. (C) Mean CT values after also normalizing based on mean thickness of each subject.

Chapter 3

3 Results

Focusing only on the data from mature hearing cats (green bars) in Figure 3.1 and ignoring group comparisons for now, we can see that the more dorsal auditory regions such as DZ and A1 tend to be thinner than their more ventral counterparts. These same trends are also present in the deaf group (purple bars), and the overall pattern of thickness values in auditory cortex is comparable between the two groups. Across hemispheres both groups show bilaterally homologous regions being similar in thickness to each other within group.

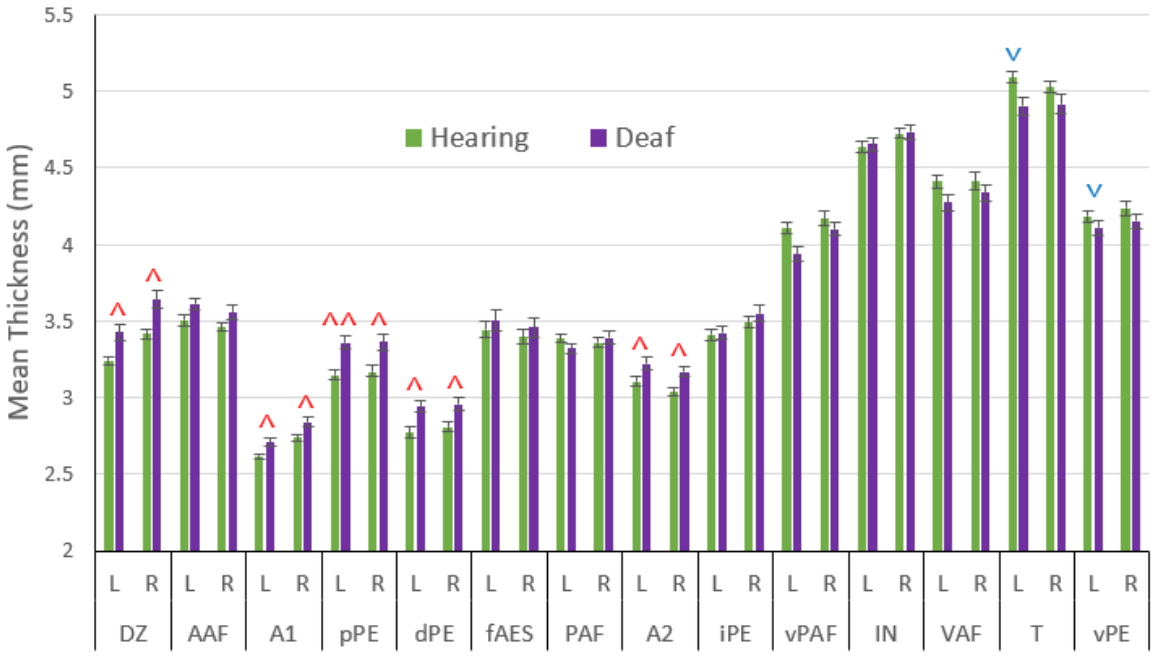

Figure 3.1 Mean cortical thickness values for each auditory region. Regions sorted based on dorsoventral position in the brain, with more dorsal regions being on the left. Downward pointing arrows indicate significant decreased thicknesses in the deaf group and upward pointing arrows indicate increased thickness. Data presented as mean + SEM. ^$p < 0.05$, ^^$p < 0.01$ between the hearing and deaf conditions.

3.1 Regression of Age and Mean CT

Before processing the raw thickness values for analysis, the mean thickness of each subject was calculated, and an effect of age was clearly identified (Figure 2.3A). As subjects ranged in age from ~6 months to nearly 6 years old, this effect had to be removed to allow for pooling of data. The effect of age was regressed out on a per-region basis to account for differences in growth over time (Figure 2.3B). After regressing out these growth slopes and mean CT for each subject (Figure 2.3C) it was possible to run all future analysis on the full dataset.

3.2 Bilaterally Thicker Regions in the Deaf Group

While comparable, the regional thicknesses aren't all the same between the two groups. Of the 14 bilateral (28 total) regions examined in this study, 10 regions were significantly thicker in the deaf group compared to the hearing group (Table 3.1, Figure 3.1). These regions were primary auditory cortex (A1L p=0.020, A1R p=0.033), second auditory cortex (A2L p=0.034, A2R p=0.015), dorsal zone (DZL p=0.015, DZR p=0.015), posterior ectosylvian auditory cortex, dorsal division (dPEL p=0.015, dPER p=0.021), and posterior ectosylvian auditory cortex, posterior division (pPEL p=0.006, pPER p=0.015). For these regions, there was no clear effect of hemisphere on the amount of thickening, meaning that the increased thickness over hearing controls was symmetric.

3.3 Thinner Regions in the Deaf Group

While all regions that were observed to be thicker in the deaf group were thicker bilaterally, there was significant thinning in the left hemisphere, and a trend toward thinning on the right (Table 3.1). These two regions are the temporal cortex (TL p=0.022, TR p=0.229) and the ventral division of the posterior auditory field (vPAFL p=0.022, vPAFR p=0.434).

Region	Left Δ (mm)	Right Δ (mm)	p Left	p Right	Notes
DZ	-0.187	-0.223	***0.015**	***0.015**	Bilaterally thicker in deaf
AAF	-0.105	-0.096	0.109	0.132	No change
A1	-0.094	-0.104	***0.020**	***0.033**	Bilaterally thicker in deaf
pPE	-0.215	-0.191	****0.006**	***0.015**	Bilaterally thicker in deaf
dPE	-0.169	-0.151	***0.015**	***0.021**	Bilaterally thicker in deaf
fAES	-0.058	-0.063	0.572	0.502	No change
PAF	0.069	-0.032	0.157	0.595	No change
A2	-0.117	-0.134	***0.035**	***0.015**	Bilaterally thicker in deaf
iPE	-0.010	-0.058	0.897	0.482	No change
vPAF	0.174	0.070	***0.022**	0.434	Left thinner in deaf
IN	-0.017	-0.008	0.812	0.897	No change
VAF	0.138	0.076	0.105	0.467	No change
T	0.185	0.112	***0.022**	0.229	Left thinner in deaf
vPE	0.072	0.083	0.365	0.365	No change

Table 3.1 Thickness changes across all auditory regions of interest in each hemisphere. Data presented as mean thickness of deaf group minus mean thickness of hearing group in mm. Regions sorted based on dorsoventral position, with DZ being the most dorsal and vPE being the most ventral. 12 regions were found to be significantly different between the groups out of the 28 tested. P-values are adjusted for false discovery rate. *$p < 0.05$, **$p < 0.01$ between the hearing and deaf conditions.

3.4 Dorsoventral Axis Correlation

Along with the trend of more dorsal regions being thinner in both groups than the more ventral regions, there was also a significant correlation between the thickness differences between the groups and the dorsoventral position of each ROI. The stereotaxic coordinates of the centroid of each ROI were calculated and then plotted against the thickness differences between the groups (Figure 3.2). The more dorsal regions in auditory cortex were thicker in the deaf group while the more ventral regions were thinner. This correlation was found to be highly significant, with $R^2 = 0.7579$ and $p < 0.00001$. No significant anteroposterior or left/right correlations of this kind were identified.

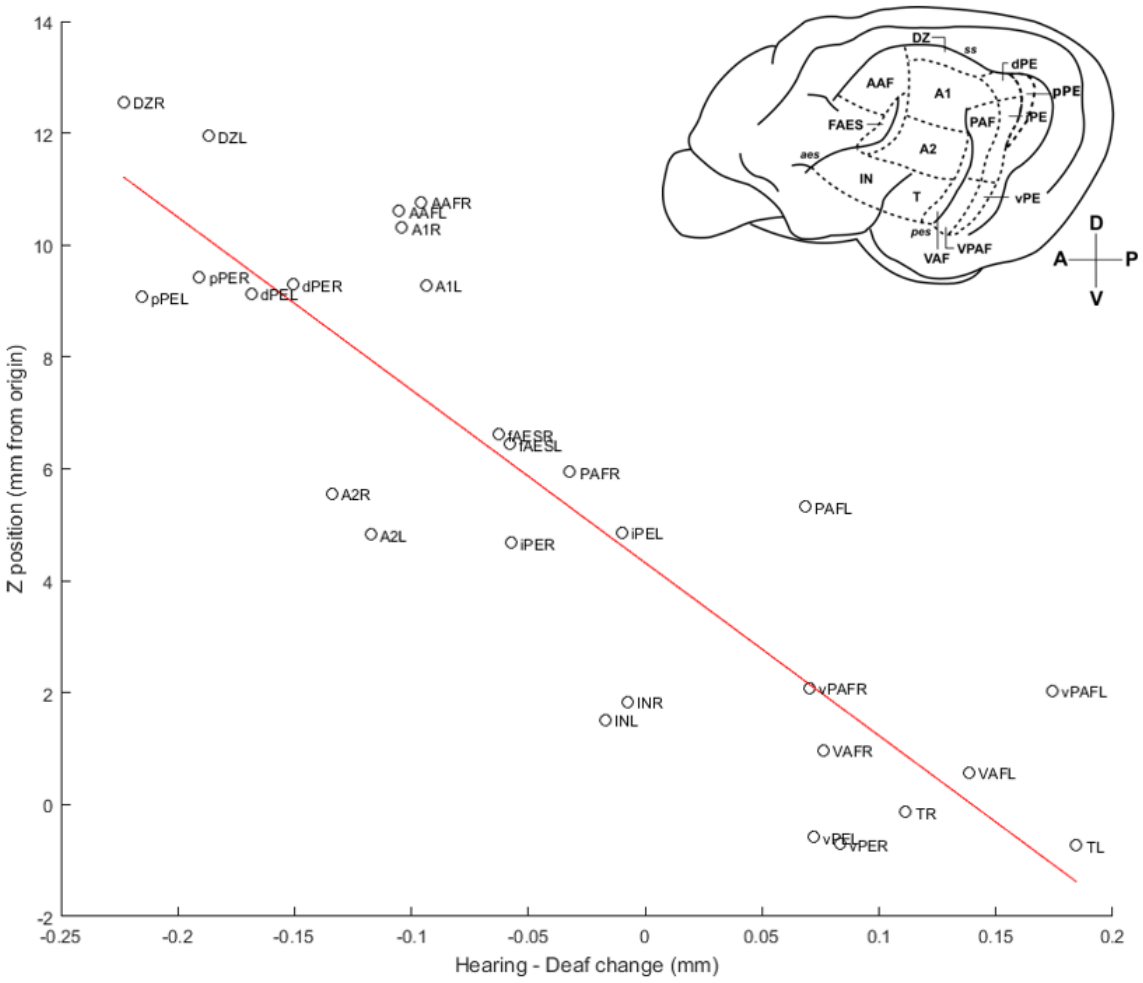

Figure 3.2 Thickness change versus vertical position of auditory regions of interest (ROIs) in the brain. Thickness change was calculated as mean thickness of each ROI in the hearing group minus mean thickness in the deaf group. A linear line of best fit has properties $R^2 = 0.7579$, $p < 0.00001$. This means that the higher in the brain a region is, the more likely that it is thicker in the deaf group and vice versa. Image in top right is a lateral view of the left hemisphere of the cat brain with auditory regions labelled.

Chapter 4

4 Discussion

The purpose of this study was to quantify the differences in auditory cortex thickness between hearing and deaf cats using MR images. In general, more dorsal regions in the hearing and deaf groups tended to be thinner than more ventral regions (Figure 3.1). Comparing the hearing and deaf groups, 12 regions were found to be significantly different (10 thicker in deaf, 2 thinner). The physical locations of the thicker and thinner regions in the deaf group are clustered, with the thicker regions being the more ventral ones. This clustering caused a very significant correlation to emerge when the dorsoventral position was examined in the brain of each ROI and the thickness change across groups (Figure 3.2). Age was found to have a very large effect on different cortical regions within each group (Figure 2.3), but effects of gender and group (hearing or deaf) were not found to be significant.

4.1 Age Regression and Consequences of Normalization

Cortical thickness decreases over the lifespan such that an animal's age at imaging had a significant effect on the mean cortical thickness across the cortical regions examined; As shown in Figure 2.3, this effect was largely driven by the youngest animals studied. As a result of this, age regression was performed on a region-by-region basis within each group. For every individual region, all subjects mean CT was plotted against age of the animals and a first-degree line of best fit was fitted to the data. The slope and intercept of this line were then used to modify each subject's measured CT value at each region based on their age. While this method allows for the use of subjects across a wide age range, it introduces a possible confound. As all subjects in the deaf group were deafened at the same age, an older deaf cat in this study will have lived longer without the use of its hearing. A longer period of deafness would likely cause a larger, or even different, change in regional cortical thicknesses across the cerebral cortex. These differential changes based on the length of deafness could be obscured by this simple age regression method. To address this issue, future work could be done to assess normal age-related cortical thickness changes in healthy cats across as wide an age range as possible. Those results could then be used to address age-related effects in this and any other cat cortical thickness work.

After this the mean CT of each subject was found and this value was used to bring all mean CTs to the population mean between both groups. Doing this type of preprocessing means that the actual thicknesses as measured by the ANTs software platform are not the ones used in the analysis, and we cannot presume absolute CT measurements from the data. Instead it allows for the comparison of CT values between the two groups in a relative sense only. Absolute thickness measurements are not the focus of this study, so this does not alter the results as presented but is important to keep in mind when normalizing data.

4.2 What Causes Thickness Changes?

As previously mentioned, cortical thickness as measured by MRI is influenced by many things including myelin content, cell type and density, and neuropil density. It is not currently known what all of the variables that go into CT are, but there is evidence to suggest that a thicker region in an MR image has more abundant inbound connections than the same region that is thinner in a different subject or group (Wagstyl, Ronan, Goodyer, & Fletcher, 2015). These connections can be from any region in the cortex, auditory or otherwise, as well as from the thalamus. With very little information coming from auditory thalamus to any auditory region in the deaf group, the increase in thickness can be understood as an increase in corticocortical connections. From previous work (Lomber et al., 2010; Meredith & Lomber, 2011), we know that some regions of auditory cortex can undergo cross-modal plasticity and be repurposed in the deaf brain. These same researchers also demonstrated an increase in dendritic spine density in fAES of ototoxically-deafened cats (Clemo, Lomber, & Meredith, 2016). Any new role most likely requires the same auditory cortical connections to perform any calculations it requires, as well as the additional strength of already existing connections from other modalities, such as vision. This does not mean that there would be more abundant connections from visual areas, but that the already existing connections are strengthened through an increase in synaptic density in the auditory region. In the case of a thinner region this would mean a possible decrease in synaptic density, or an increase in myelin content in that area (Sowell, Thompson, Holmes, Batth, et al., 1999; Sowell, Thompson, Holmes, Jernigan, & Toga, 1999; Sowell, Thompson, Tessner, & Toga, 2001). This explanation accounts for the lack of significant results from retrograde tracer studies in deaf cat auditory cortex and suggests that, perhaps, when comparing plasticity in connections after sensory loss, anterograde tracers could allow for a more interesting and complete story.

4.2.1 Is This Unmasking?

What is being described above is the theory of unmasking of non-auditory sensory inputs in the absence of auditory input. This theory implies that a given region could take over or enhance a given behaviour if it already possesses the required inputs and outputs to influence that behaviour, thus requiring no new connections. These already existing connections would be used for subthreshold or multisensory processing in the normally developed brain, but in the case of sensory deprivation, they become the only remaining inputs to that region (Bavelier & Neville, 2002; Kato, Price, Ferrer, & Blakemore, 1993). This theory could account for behavioural and anatomical data from deaf cats; not only do the non-auditory inputs appear to change very little in number, if at all, but the new behavioural roles of regions appear to be related to their roles in audition in the hearing brain. This idea is also supported by the idea that the brain is likely adapted to make behavioural judgements by consolidating evidence across multisensory inputs. Very rarely in the real world is a percept comprised of a single sensory modality, which means that an efficient brain would combine multiple unisensory percepts together into one more salient multisensory percept. In any brain region where information from one sensory modality is influenced by relevant information from another, unmasking is possible if one of those senses is lost. For a more in-depth review of this theory, see (Dormal & Collignon, 2011).

4.3 Retrograde Tracer Studies versus Cortical Thickness

Many retrograde tracer studies have examined the structural connections between regions of the cat auditory cortex and the rest of the brain (Lee et al., 2004; Lee & Winer, 2005, 2008a, 2008b, 2008c, 2011). While these studies show very little changes in terms of afferent connections to auditory cortical regions following deafness, we still find CT changes in these same regions between the hearing and deaf group in our present data. This backs up the idea that GM CT changes are not caused by changes in overall cortical connectivity, but more likely affected by the local synaptic density and dendritic branching. As stated previously, there are many characteristics of GM that contribute to CT, so there are very likely other variables affecting the current measurements. Continuing this kind of work, it would be very beneficial to add myelin and/or diffusion tensor imaging to the analysis to further describe the changes that occur after sensory deprivation.

4.3.1 Ex Vivo Cortical Thickness Comparisons

The thickness of three regions of cat auditory cortex (A1, A2, and DZ) have been measured histologically post mortem in hearing and congenitally deaf cats (Berger et al., 2017). A1 and A2 were found to be thinner in the deaf, an effect attributable to thinning of layers IV-VI. Based on these results we would expect at least these three regions to be thinner in our data, but that is not the case. In fact, A1, A2 and DZ were all found to be significantly thicker in the deaf in the current study. The reason for the differences in these results is unknown, but there are some things to consider when comparing these results. The first is that the current work used ototoxically-deafened animals, whereas Berger used a congenital deaf cat model. While both experimental groups experience deafness, results from cytoarchitectonic studies involving congenitally deaf animals show noticeable differences in AC region boundaries and connectivity (Barone, Lacassagne, & Kral, 2013; Kral, Schröder, Klinke, & Engel, 2003; Kral, Tillein, Heid, Hartmann, & Klinke, 2005; Wong, Chabot, Kok, & Lomber, 2014). There are also the differences in methodology used between this current work and that of Berger. This work is centered around in vivo measurements obtained using 0.5mm voxels in 55 subjects, as opposed to Berger's congenital deaf work which analyzed the fixed tissue of 8 subjects under a light microscope. Although light microscopy allows for more precise measurements and intrasubject parcellation of cortical regions, there are some significant caveats to this technique. Ex vivo measurements of cortical tissue require that the tissue be fixed to prevent damage during mounting and staining. This fixation can cause nonuniform shrinkage of the tissue, altering the reported thickness. In contrast, MRI scans can be obtained for living tissue thus avoiding fixation and the issue of shrinkage. This neuroimaging technique is also non-invasive, and thus can also be performed repeatedly on each subject. MRI-derived thickness is an efficient method that reduces the number of terminal subjects in a study while maintaining the potential of a large n and high power.

4.3.2 Behavioral Functional Plasticity and Thickness

Experience-dependent changes in GM thickness have been documented in humans, with studies focused on topics ranging from musicianship and amusia (Bermudez et al., 2009; Hyde et al., 2007), to memory (Engvig et al., 2010; Metzler-Baddeley, Caeyenberghs, Foley, & Jones, 2016), meditation (Lazar et al., 2005), attention-deficit/hyperactivity disorder (Shaw et al., 2006), and even playing video games (Kuhn et al., 2014). The breadth of CT research is not limited to humans,

and there has also been some work in mice (Lerch et al., 2011). These studies show that the brain adapts quite quickly to new experiences, at a level that can be measured by MRI. What these studies don't agree on is what is better: thicker or thinner cortex? Thinner cortex has been argued to be possibly more efficient considering the musician work by the Zatorre group (Bermudez et al., 2009; Hyde et al., 2007), but other work suggests that a thicker cortex develops from excess use (Kuhn et al., 2014; Lazar et al., 2005). In the present work, the "experience" is a lack of auditory input over the course of months or years and results in both thicker regions (the majority in this study) and thinner regions, all of which reside in what was previously auditory cortex. Behaviourally we already know that at least some of these regions have new uses in the sensory-deprived brain of the cat (Lomber et al., 2010), but there is no correlation between behavioural plasticity and the new CT of AC.

4.4 The Dorsoventral Trend

From Figure 4.1 it is clear that there is some sort of correlation between the dorsoventral location of any given AC region and thickness changes following deafness. The more dorsal a region is, the thicker it will be relative to the control group. This correlation is highly significant ($p<0.00001$) and could be due to the fact that regions with similar roles to one another are often near each other or even share a border. This proximity effect forms clusters of regions with similar tasks that allows for axial correlations to emerge. One such clustering of AC regions is that of the auditory "what" and "where" pathways. These two groups contain regions that are specialized to process either spectrotemporal features of sound or the sound's origin in space. Interestingly, the "where" pathway regions are, as a group, more dorsally located than the "what" pathway regions (Figure 1.2). Lining this up with our dorsoventral correlation result, it would appear that there is a possibility that what we are actually quantifying is a thickness change difference between the two auditory processing stream regions. Because of the type of auditory, visual, and somatosensory information going into the where pathway differs greatly from the type of information going into the what pathway, it is understandable that these two groups of regions adapt very differently when auditory input is removed.

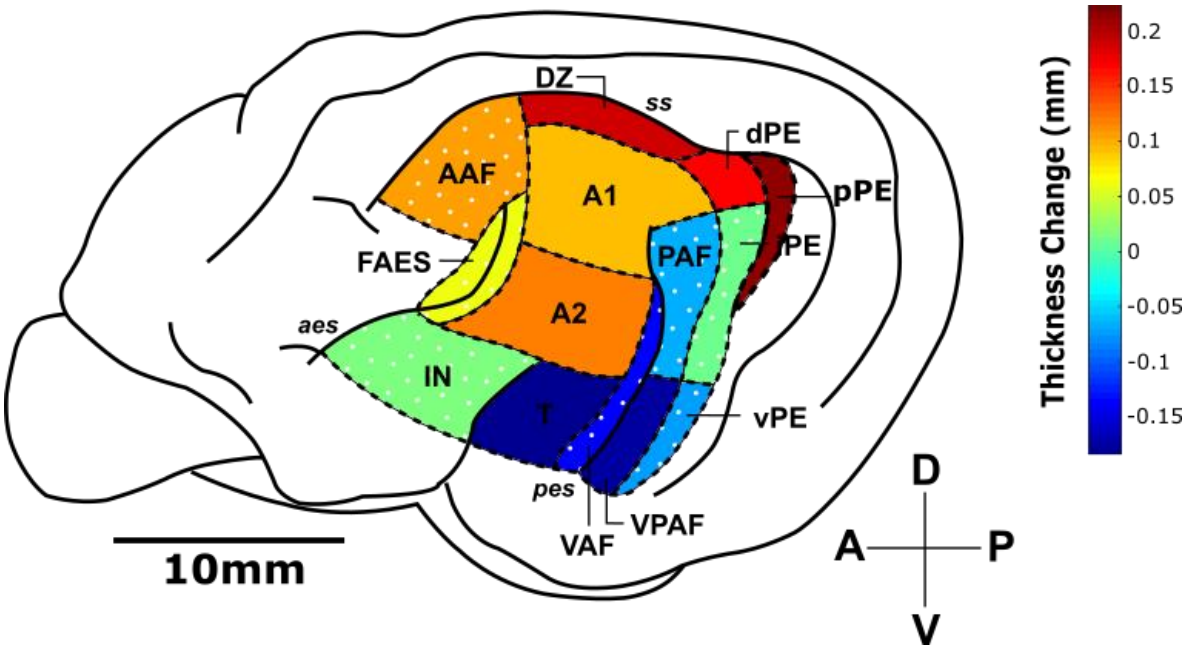

Figure 4.1 Visual representation of thickness changes between the two groups. Data presented as mean thickness of deaf group minus mean thickness of hearing group in mm on the left hemisphere. Significant changes are denoted by solid colours, while nonsignificant changes contain white dots. The dorsal zone (DZ), dorsal and posterior divisions of posterior ectosylvian auditory cortex (dPE, pPE), primary auditory cortex (A1), and second auditory cortex (A2) were all significantly thicker in the deaf group. Temporal auditory cortex (T) and the ventral division of the posterior auditory field (VPAF) were found to be thinner.

4.5 What/Where Pathways

There are many factors that come together to influence the CT of any cortical region. Sometimes a region will become thinner with heavy use (Hyde et al., 2007), and at other times it seems that a well-used region will become thicker (Maguire et al., 2000). With the present data, the only thing that can be said about these collections of regions is that the where pathway regions probably have a different adaptation to deafness than the what pathway regions. Their perceived increased CT would most likely stem from increased synapses from non-auditory parts of the brain and a maintenance of neuronal cell size. In contrast, while it is unlikely that the what pathway regions are no longer used at all, they don't appear to just have increased the amount of information coming in from other brain regions. It is possible that these regions already had the number and type of connections that they needed to continue on with their new tasks and became thinner because of the auditory thalamic and related connections disappearing from non-use. Without more anterograde tracer studies or a different MR contrast this is all just speculation based purely on MRI-derived CT.

4.6 Future Directions

The results of this work do not necessarily line up with past work in cats (non-MRI) or humans. To determine the cause of these discrepancies, further work could be conducted. All of the present findings were obtained from MR images of hearing and deaf cats. While MRI allows for in vivo estimation of cortical thickness, ex vivo measurements of brain slices such as in the Berger work (Berger et al., 2017) gives a much higher measurement resolution. There is a tissue shrinkage issue that arises, but this can be lessened, even down to <4% shrinkage as in that study. The curvature of the brain also impacts the effectiveness of this method, as slices in each individual brain can only be obtained in one plane. To alleviate this, three-dimensional surfaces could be reconstructed from tracings on each slice, and thickness values would be extracted from these surfaces. This can even be done on many of the subjects of this work which could validate the MR measurements.

A different technique that could be used to explain conflicting results is diffusion-weighted-imaging(DTI)-based probabilistic tractography. This method involves obtaining a different type of MR image and using it to estimate the direction of axonal bundles in the white matter of subjects. These estimations are then used to calculate probable region-to-region connectivity matrices.

Differences in the matrices between groups gives insight into possible reweighting of connections between regions, giving more insight into the causes of thickness differences.

4.7 Conclusions

Differential thinning and thickening of distinct regions of auditory cortex was observed in the deaf cat relative to normal hearing controls. In general, it was observed that regions of the “where” auditory pathway were thicker in deaf subjects when compared to controls, and “what” pathway regions were thinner (in the left hemisphere) or were no different than in controls (right hemisphere). This effect may be due to differences in the extent to which these two processing streams undergo cross-modal plasticity, potentially due to the type of information processed in each and/or the connections between modalities that already exist in the hearing brain. What is clear is that a great deal is changing in the sensory-deprived cortex which has not been captured in previous anatomical studies that relied upon retrograde tracers to quantify projections between cortical regions. Collectively, these track-tracing studies and the current MR-based quantification suggest that even though this animal model does not undergo large connectivity changes following deafness, the auditory cortex still experiences anatomical changes that are expressed as regional thickness changes that can be measured in vivo. Future work will need to be done to assess the exact cause of these changes.

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auditory cortex following early- and late-onset deafness. *Cerebral Cortex*, *24*(7), 1778–1792. https://doi.org/10.1093/cercor/bht026

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Curriculum Vitae

STEPHEN GORDON

EDUCATION

2011-2016 – BMSc Honors Specialization Medical Sciences, Western University Canada
Dean's Honor List
Area of Specialization: Anatomy and Cell Biology
Fields Included: Microbiology, Immunology, Pathology, Ethics, Animal Models
Included some computer science programming courses

2006-2010 – Sir Frederick Banting Secondary School
Honor Roll
University-level courses in calculus, biology, chemistry, physics, French
AP French Test mark of 5.0
French language certificate

RESEARCH

2016-Present – Graduate Student, Cerebral Systems Lab
Experience with cats and NHPs in relation to behavioural tasks and husbandry
Current projects involve structural MRI
Development and refinement of new project setups

2016-2019 – Teaching Assistant, Neuroscience 2000
Running laboratory sessions with Backyard Brains equipment
Proctoring exams and marking assignments

2013-2016 - Volunteer/Student Technician, Cerebral Systems Labs
Stained tissues to be viewed under microscopy
Trained animals to do a behavioral task related to audition
On-the-job training pertaining to microscopy and MATLAB

Aug 2014-Aug 2015 – Student Microbiology Laboratory Technician, AAFC
Culturing a variety of microorganisms from environmental samples
Aseptic technique in a CL-2 laboratory
Writing code used for bioinformatics projects pertaining to sequencing
Identification of unknown microorganisms
Archiving of thousands of identified bacterial isolate samples

PUBLICATIONS

Lau, C. H.-F., van Engelen, K., Gordon, S., Renaud, J., & Topp, E. (2017). Novel Antibiotic Resistance Determinants from Agricultural Soil Exposed to Antibiotics Widely Used in Human

Medicine and Animal Farming. *Applied and Environmental Microbiology*, *83*(16), 1–18. https://doi.org/10.1128/aem.00989-17

CONFERENCE PRESENTATIONS

Gordon SG, Stolzberg D, Lomber SG. 2016. The ventriloquism aftereffect in the cat. Poster presentation at the auditory Gordon Research Conference in Lewiston, Maine.

Gordon SG, Stolzberg D, Lomber SG. 2017. The ventriloquism aftereffect in the cat. Poster presentation at the International Multisensory Research Forum in Nashville, Tennessee.

Gordon SG, Butler BE, Lomber SG. Cortical thickness measurements in hearing and deaf cats using ultra high-field magnetic resonance imaging. Poster presentation at the Resting-State and Brain Connectivity conference in Montreal, Quebec.

Gordon SG, Butler BE, Lomber SG. Cortical thickness measurements in the hearing and deaf cat using high-resolution Magnetic Resonance Imaging. Poster presentation and the Blind Brain Workshop in Lucca, Italy.

Gordon SG, Butler BE, Lomber SG. Differential Thinning and Thickening of Auditory Cortex in Deaf Cats Revealed with Ultra-High-Field MRI. Poster presentation at the International Hearing Loss Conference in Niagara-on-the-lake, Ontario.