11-26-2018 1:00 PM

Medial Geniculate Projections to Auditory and Visual Cortex in Hearing and Deaf Cats

Benjamin Trachtenberg
The University of Western Ontario

Supervisor
Lomber, Stephen G.
The University of Western Ontario

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

Abstract

Following early-onset deafness, studies have demonstrated crossmodal plasticity, throughout “deaf” auditory cortex. Crossmodally reorganized auditory cortex shows increased dendritic spine density, which suggests increased numbers of axon terminals. I examined projections from the medial geniculate body (MGB) of hearing and early-deaf cats in order to reveal the distribution of synaptic boutons in the cortex, originating from MGB neurons. Anterograde fluorescent dextran tracers were deposited bilaterally in the MGB in order to label cortical axon terminals. Deafness resulted in axon terminal increases in visual cortex, and conservation of auditory axon terminal distribution. Visual areas PLLS and area 18 received increased projections from deaf MGB. Distributions of thalamocortical axon terminals in crossmodally reorganized deaf auditory areas PAF, DZ and fAES were stable. These findings indicate a need for studies of corticocortical connectivity, to find an anatomical basis for crossmodal reorganization.

Keywords: Early-onset deafness, cross-modal reorganization, anterograde pathway tracer, medial geniculate body (MGB), thalamocortical connectivity

Statement of Co-Authorship

The experiment, data collection, analysis, and writing of this thesis were conducted primarily by the thesis author, Benjamin Trachtenberg. Dr. Blake Butler assisted in experimental design and initial analysis of data. Dr. Stephen Lomber assisted with all aspects in the completion of this thesis, and provided advice and editing for all sections of the thesis.

Acknowledgements

Thank you to my supervisor, Dr. Stephen Lomber, for his guidance and support. I am grateful for his insightful ideas on my research throughout my degree. I appreciate his creativity in problem solving, and his attention to detail. I am honoured to have studied and worked under his guidance.

I would like to thank the members of the Cerebral Systems Lab for their support and help through this process. I especially want to thank Dr. Blake Butler for his help with many aspects of this project, and for the mentorship he provided at all levels of my research. His guidance was invaluable to the technical work I have done.

Thank you to my advisors Dr. Brian Corneil, Dr. Brian Allman, and Dr. David Sherry, for their expertise and coaching. They were always willing to help and provide necessary feedback on the project, without which I could not have finished this work.

I would like to humbly thank my parents, Ida and Mark Trachtenberg, family, friends, and Arian for their continual positivity and encouragement in this process.

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A1	Primary auditory cortex
A2	Second auditory cortex
AAF	Anterior auditory field
ABR	Auditory brainstem response
AEV	Anterior ectosylvian visual area
ALLS	Anterolateral lateral suprasylvian area
AMLS	Anteromedial lateral suprasylvian area
DLS	Dorsal lateral suprasylvian area
dPE	Dorsoposterior ectosylvian gyrus
DZ	Dorsal zone of auditory cortex
EEG	Electroencephalography
EPp	posterior aspect of the posterior ectosylvian gyrus
fAES	Auditory field of the anterior ectosylvian sulcus
fMRI	Functional magnetic resonance imaging
IN	Insular auditory cortex
iPE	Intermediate division of the posterior ectosylvian auditory cortex
LGN	Lateral geniculate nucleus
MEG	Magnetoencephalography
MGB	Medial geniculate body
PAF	Posterior auditory field
PLLS	Posterolateral lateral suprasylvian area
PMLS	Posteromedial lateral suprasylvian area
SSG	Suprasylvian gyrus
SSS	Suprasylvian sulcus
T	Temporal auditory area
V1	Primary visual cortex

VAF	Ventral auditory field
VLS	Ventral lateral suprasylvian area
VPAF	Ventral posterior auditory field
vPE	Ventral portion of the posterior ectosylvian auditory cortex
%	percent

Chapter 1

Introduction

1.1 Hearing Loss

Hearing loss affects 466 million people, including 34 million children, with the number of affected individuals increasing every year. Hearing loss can be incurred at a number of different stages in life, and due to a number of factors. Individuals can be born deaf (congenital deafness) or experience a period of normal hearing before the onset of deafness. Deafness can be caused genetically, as well as through complications at birth, untreated infectious diseases, excessive noise exposure, and normal ageing (WHO, 2018).

Hearing loss is defined as the inability of an individual to hear sounds at or below 25 decibels, in one or both ears (WHO, 2018). Deafness can be defined as the degree of hearing loss at which sounds cannot be heard, even when dramatically amplified. Profound deafness is observed in individuals unable to hear sounds below 91 dB, while total deafness is observed in individuals completely unable to hear any sound (Elzouki, 2012).

Those who experience hearing loss can rely on hearing aids or learn sign language in order to facilitate communication and daily living activities. The development of cochlear implants in the 1950s has enabled some individuals to regain auditory input. Cochlear implants allow for perception of sound through bypassing the normal auditory pathway. Implants include an external receptor for auditory stimuli and an internal processor, surgically implanted, which delivers electrical stimulation to the auditory nerve, and restores metabolic activity in the auditory cortex (Wilson and Dorman, 2008;

Naito et al., 1997). At the same time, clinical outcomes for cochlear implant users are highly variable. The degree to which the implant is successful largely depends on the timing of the implantation. It has been proposed that implantations occurring earlier in life are more successful at restoring hearing (Ganek et al., 2012).

The increase in metabolic activity of auditory cortex can also result from the process of cross-modal reorganization, in which deprived areas of the brain (ie. auditory cortex in deaf individuals) are able to process information from the remaining senses (ie. sight and touch). Following this process of reorganization, the auditory cortex will no longer be able to respond to the implant. Once cross-modal reorganization has occurred in a deaf brain, the inability of auditory cortex to respond to cochlear implants indicates the possibility that the auditory cortex is permanently rewired in deaf patients. Thus, the profound benefits that cochlear implants permit to deaf individuals are limited both by the duration of deafness, and by the ability of the auditory cortex to reorganize following this sensory deprivation. This is due, in part, to the altered sensory information carried through a deaf auditory cortex, which has been reorganized to process other sensory information. As a result, the question remains if there is a critical period to provide sound to the auditory cortex (Litovsky, 2015).

1.2 Auditory Pathway in Hearing Subjects

In order to study the consequences of dysregulation (i.e. changes due to sensory deprivation) of sensory systems, it is necessary to first understand the hearing, intact condition. As defined by Schnupp and colleagues (2011), hearing is an “everyday miracle”, through which a complicated cascade of neurophysiological events allows perception and discrimination of sound. The auditory system creates a perceptual space,

in which sounds can be grouped, segregated, and analyzed to promote language and communication (Litovsky, 2015). Sounds have varying properties and are processed differently. However, healthy young humans can typically hear sounds in the range of 20-20000 Hz (Cutnell et al., 1998). The auditory pathway can thus be examined through its anatomical and developmental features.

Normal hearing occurs due to detection of vibrations in the environment, localized to the membranes of the cochlea, allowing perception of sound (Plack, 2014). Hearing is initiated before birth, as fetal heart rate responses change due to sound presentation. At approximately 27 weeks gestation, an auditory brainstem response (measuring brainstem and eighth cranial nerve activity), can confirm the presence of hearing (Galambos and Hecox, 1978). Further, in classical habituation tasks, fetuses at 35 weeks gestation can perceive novel auditory stimuli (Shahidullah and Hepper, 1994). The auditory system has been found to be highly plastic during development, as postnatal change and growth occur. The ear canal increases in length and diameter during early (first 2 years postnatal) life (Keefe et al., 1993). Anatomical changes during physical development are thus important in refining signal transduction of auditory stimuli, and corresponding processing in the auditory system. In the inner ear, cochlear development typically follows a frequency gradient organization, high to low, from basilar membrane to apex (Pujol and Hilding, 1973). Following birth, the auditory system undergoes rapid changes. Within the first 6 months of life, high-frequency tone discrimination is finely tuned, and infants perform similarly to adults on frequency discrimination tasks by age 1 (Olsho et al., 1987, 1988).

In order to make sense of the world around them, and communicate effectively, infants must master the ability to process sounds in their environment. Just as infants need to discriminate between different sounds, they also need to discriminate between relevant and irrelevant sensory information. In fact, infants have a remarkable ability to discriminate voices similar to their mother's, from background noise (Barker and Newman, 2004). Further, it has been proposed that infants make use of multisensory integration, such that localization of auditory stimuli promotes orienting of the head in order to bring the stimulus into view (Litovsky, 2015). In animal models, this auditory localization has been found to be processed in the auditory cortex (Nodal et al., 2010, 2012).

1.3 Review of Reorganization

It has been suggested that postlingually deaf individuals (hearing loss after acquisition of speech and language) retain a normally developed auditory cortex, while prelingually deaf individuals (i.e. congenitally deaf) do not (Kral and Eggermont, 2007). The ineffectiveness of cochlear implants in some individuals can be explained by the phenomenon of visual "take-over" of auditory cortical areas, such that the cross-modal reorganization induced by sensory deprivation (deafness) cannot be reversed (to undertake normal auditory processes) in cochlear implant users (Stropahl, 2017).

The cellular explanation for the variability of cochlear implant outcomes is based in metabolic activities in the cortex. It has been shown that the implantation of cochlear devices actually increases metabolic activity in the auditory cortex (Naito et al., 1997). In a critical study in the cellular and anatomical field of neuroplasticity, Lee and colleagues demonstrated that the decreased metabolic activity in deaf auditory cortex can be restored

through cross-modal reorganization in prelingually deaf patients, and that the auditory cortex has difficulty in processing input from cochlear implants (Lee et al., 2001). Using positron emission tomography, Lee and colleagues discovered that the degree of hypometabolism before implantation was related to the potential for hearing improvement after implantation (Lee et al., 2001). It was further found that duration of deafness decreased the total hypometabolic area in auditory cortex. As proposed elsewhere, the changes in metabolism, accompanied by "permanence" of extra-auditory perception in auditory cortex, may be due to alterations in the afferent networks of visual and somatosensory systems (Rauschecker, 1995). Therefore, taken together, it can be seen that the auditory system is limited in its capacity for restoration following deafness. As summarized, the functions of the deaf auditory cortex are usurped by those related to cross-modal reorganization, allowing superior visual and tactile performance on behavioural tasks, while hindering the effectiveness of cochlear implants, and the recovery potential which they bestow (Lee et al., 2001).

1.4 Effects of Deafness on the Auditory Pathway

Deafness has a considerable effect on the nervous system. The auditory pathway itself is drastically altered, as large-scale degeneration occurs (Shepherd and McCreery, 2006). In the peripheral auditory pathway, neural ganglion cells are found to significantly degenerate in structure and function following deafness (Leake et al., 1999). As well. cortical maps, which allow for the remarkable sound discrimination in infants, are lost following early profound deafness (Kral et al., 2009). In this section, the physiological effects of deafness on the auditory system are discussed.

The auditory pathway extends from the outer ear to the cerebral cortex, and undergoes vast refinement due to sensory experience, or lack thereof (Eggermont, 2008). As discussed previously, the causes of deafness are varied, and can be modelled genetically, physically, or ototoxically (Butler and Lomber, 2013). The effects of deafness have various anatomical outcomes, which all typically culminate in the destruction of cochlear hair cells (Leake et al., 1997; Ryugo et al., 1998). From the cochlea, auditory signals are normally passed to the cochlear nucleus in the brainstem, via spiral ganglion neurons (Sento and Ryugo, 1989). In deaf animals, these spiral ganglion neurons are greatly reduced in number, with the most profound decreases found in congenitally deaf animals, as well as those who have experienced deafness for an extended period of time (Ryugo et al., 1998; Shepherd et al., 2004). The cochlear nucleus itself exhibits decreases to neuronal volume and number, resulting in an overall decrease in size of the entire area (Hardie and Shepherd, 1999; Nordeen et al., 1983). Consequences of these reductions in congenitally deaf animals include an absence of spontaneous activity in the cochlear nerve, thus affecting the auditory pathway beyond this point (Leao et al., 2006).

Further in the auditory pathway, the cochlear nucleus projects to the inferior colliculus. Although there is ample evidence of degradation in the cochlea and cochlear nucleus during and following deafness, the pattern of projection from the cochlea to the auditory midbrain nucleus, the inferior colliculus, appears unchanged (Heid et al., 1997; Moore, 1990; Moore and Kowalchuk, 1988). Heid and colleagues injected a retrograde pathway tracer into the inferior colliculus of deaf white cats, and observed only minor differences in projection strength when compared to normal hearing cats. Previous

experiments had identified identical sectors of the central inferior colliculus to be activated by stimulation of particular cochlear cells in both hearing and deaf kittens. In light of this preservation, it was proposed that tonotopy is preserved at the level of the inferior colliculus in the auditory pathway (Snyder et al., 1990). While the cochlear and olivary projection to the inferior colliculus appears unchanged, the midbrain nucleus does exhibit some cellular changes due to deafness. It has been found that ototoxically early-deafened cats show decreases to both neuronal size, and to synaptic and presynaptic vesicle density (Nishiyama et al., 2000; Hardie et al., 1998).

The auditory pathway proceeds from the inferior colliculus to the thalamus. The auditory thalamic nucleus is the medial geniculate body (MGB), consisting of several subdivisions, each characterised by a distinct areal cartography, defined by differing densities and types of neurons, and by a unique projection pattern to auditory cortex (Lee and Winer, 2008). In hearing cats, Lee and Winer identified that the ventral MGB projects largely to core auditory cortex, while dorsal MGB projects to secondary/belt auditory areas, and medial MGB projects to the entirety of auditory cortex (Lee and Winer, 2008) As the last auditory nucleus preceding the cortex in the auditory pathway, the MGB has attracted considerable interest in studies of auditory deprivation. However, there have only been limited studies on deafness regarding the projections to and from the MGB. One early study, conducted by Stanton and Harrison (2000) demonstrated that the number of thalamic MGB cells projecting to primary auditory cortex (A1) is unchanged in neonatally deafened cats. In addition, this study identified no changes in the tonotopic maps conferred upon A1 by the ventral division of the MGB (vMGB). Specifically, within A1, frequency representation increases in the posterior to anterior direction (Reale

and Imig, 1980). The authors concluded that reorganization of tonotopic maps must occur at subthalamic nuclei (Stanton and Harrison, 2000). More recently, studies investigating the thalamocortical projection have been undertaken, specifically looking at cell body density in the MGB and its subdivisions. For example, stable thalamocortical projection to A1 was found in hearing, early-deafened, and late-deafened cats (Chabot et al., 2015).

Following auditory processing in the thalamus, signals are transmitted to the cortex, for higher level integration of stimuli. Primary auditory cortex is the most studied auditory cortical area. However, there is much debate surrounding the effects of deafness on A1. In separate studies by different research groups, it has been demonstrated that A1 both retains the same volume in congenitally deaf cats as in hearing cats, and that A1 is seen to decrease in volume due to an expansion of neighbouring areas (Kral et al., 2002; Wong et al., 2014). Further it has been shown in congenitally deaf cats, that A1 has decreases to number and span of dendrites, while also exhibiting increases in dendritic spine density (Clemo et al., 2017).

In spite of these contradictory findings, one might expect that the deaf auditory cortex is incapable of any sensory-driven activity. There is considerable debate on this topic. Kral and colleagues found increases in spontaneous A1 activity in congenitally deaf cats, and proposed the cause to be an increase in thalamocortical input (Kral et al., 2003). To further this theory of auditory preservation, it has been found that a basic cochleotopic organization is conserved in the primary auditory cortex of congenitally deaf cats (Hartmann et al., 1997). As a basis for this preservation, it appears that significant portions in the auditory pathway retain normal projection patterns and strength in deafness. As an example, the olivary-cochlear nucleus pathway appears defined before

the onset of cochlear activity (Kitzes et al., 1995; Russel and Moore, 1995). Taken together, deafness appears to modify much of the auditory pathway. In light of modifications such as these, the reorganization of auditory cortex in deafness is even more fascinating.

1.5 Cross-modal Reorganization in Auditory Cortex

The nervous system allows integration of environmental sensory information, such as sight, sound, touch, taste, and smell. Sensory deprivation disrupts this integration, such that input from the intact senses (for example, vision in a deaf individual) is processed more efficiently. In humans, behavioural and imaging studies have been conducted in order to assess sensory reorganization in deaf individuals. As summarized above, deafness can occur in various forms and stages, with accompanying variability in effects and clinical outcomes (Butler and Lomber, 2013). Early theories on sensory deprivation hypothesized that the loss of one sense would drastically impair normal sensory and cognitive development, as normal behaviour required full integration of all intact senses (Axelrod, 1959). However, sensory deprivation, as touched on in the above section, reveals the ability of the nervous system to adapt to its environment, such that spared senses are processed in deprived areas of the brain. This phenomenon has been called compensatory plasticity, and has been studied extensively in humans and animal models, through behavioural, imaging, electrophysiological, and anatomical techniques (Rauschecker, 1995). In fact, when one thinks about how deaf individuals interact with their environment, there is a basic need for reliance on non-auditory input. This includes

relying on visual cues for attention-orientation, as well as the use of sign language in place of spoken linguistic communication (Merabet and Pascual-Leone, 2010).

It has been shown that deaf individuals have superior face discrimination compared to their hearing counterparts (Benetti et al., 2017). Functional magnetic resonance imaging (fMRI) has allowed for extensive exploration into the nature of cross-modal reorganization. Through fMRI scanning, it has been revealed that deaf individuals display higher activity in the auditory cortex (posterior temporal sulcus) when performing face recognition tasks (Benetti et al., 2017). Earlier work by Neville and Lawson (1987) tested visual motion discrimination in hearing and congenitally deaf individuals. Although performance between groups was similar for targets located centrally in the field of vision, it was found that deaf participants performed better on this task when targets were located peripherally. In addition, event-related potentials recorded during the task were seen to be increased in the occipital lobe and left hemisphere of deaf participants, indicating a large-scale reorganization of the congenitally deaf brain (Neville and Lawson, 1987). This same group had previously identified increases in visual evoked activity in visual and auditory cortex of congenitally deaf individuals, indicating a cross-modal reorganization of auditory areas due to deafness (Neville et al., 1983).

Using magnetoencephalography (MEG), visually-induced activity has been found in the right auditory cortex of deaf individuals, but not in normally hearing individuals (Finney et al., 2003). Lambertz and colleagues studied brain activity while participants viewed sign language. It was found that deaf individuals, but not hearing or those with partial hearing loss, exhibited increased activity in auditory cortex (Lambertz et al., 2005). It has been proposed that this type of reorganization is due to Hebbian

mechanisms, such that the absence of competitive auditory input allows intact modalities (i.e. visual and somatosensory information) to have increased activity and processing capacity in multimodal cortices (Bavelier and Neville, 2002). This mechanism can be further applied to the study by Lambertz and colleagues, such that the degree of reorganization is dependant on the degree of hearing loss (more extensive loss) and would result in more extensive reorganization.

Animal models allow for a deeper understanding of the root causes of cross-modal reorganization in the cortex. In a seminal study, Lomber and colleagues found similar behavioural results in deaf cats as those reported above in humans (Lomber et al., 2010). When tasked to localize visual stimuli, deaf cats performed better than hearing cats for presentations in the periphery. Deaf cats also exhibited decreased visual motion detection thresholds, compared to normal hearing cats. The benefit of this animal model is the ability to explore anatomical manipulations, in order to test the causes of the observed cross-modal reorganization. In light of the above findings in humans, a reversible deactivation method was employed, whereby cooling loops were implanted over various cat auditory cortical areas, in order to completely eliminate action potentials in those areas (Lomber et al., 1999). Thus, animals could perform the task with a warm (normally functioning cortex), or a cool (with a specific auditory area deactivated) cortex. Areas implanted with these loops included those already discussed, such as A1, as well as the dorsal zone (DZ), and the posterior auditory field (PAF). When A1 was deactivated, no change was observed in the above visual behavioural tasks in deaf cats, who retained their superior performance compared to normal hearing cats. However, when PAF was deactivated, deaf cats lost their visual localization advantage, and performed similarly to

hearing cats. Further, when DZ was deactivated, deaf cats performed similarly to hearing cats on visual motion detection tasks (Lomber et al., 2010). It was thus concluded that visual processing was occurring in what would normally be auditory cortex, with the visual processing being localized to specific areas of auditory cortex.

Interestingly, the reorganized auditory areas appeared to conserve their function, while switching modality. DZ of auditory cortex is normally involved in auditory motion processing. In the deaf cats, this function appeared to be preserved, while the modality of sensory processing switched from auditory to visual input. This indicates a selectivity of cortical neurons for a function, rather than a specific modality. It is important to note that while there is controversy surrounding a core auditory area such as A1, higher order areas like DZ, PAF, and the anterior ectosylvian sulcus (fAES) appear to reorganize more readily (Meredith et al., 2011).

1.6 Anatomical Basis of Cross-modal Reorganization

The behavioural, functional, and electrophysiological bases of cross-modal reorganization have been well studied, resulting in a comprehensive literature on the deprivation-induced compensation undertaken by the auditory cortex (Merabet and Pascual-Leone, 2010). However, the anatomical basis for these changes is still poorly understood. The study of anatomical changes due to deafness has grown substantially in the last five years, in part due to the valuable finding that SMI-32 antibody staining allows for clear delineation of cortical borders in the cat brain (Mellott et al., 2010). This has facilitated research into the composition of individual cortical regions in hearing and deaf cats. Through the SMI-32 protocol, neurofilament proteins in the cortex are stained

with characteristic intensities, such that a border can be identified between neighbouring areas, based on intensity of staining of cells throughout cortical layers. Using SMI-32 staining profiles, Wong and colleagues measured the volume of individual auditory cortical areas in deaf cats. It was found that A1 decreased in volume in early deaf cats, with resultant increases in the neighbouring second auditory cortex (A2). It was proposed that this change was due to a dorsal shift in A1/A2 border, as well as a ventral shift in the A1 border with visual and somatosensory areas (Wong et al., 2014). This indicates a clear anatomical difference between deaf and normal hearing auditory cortex.

Another branch of anatomical study in models of sensory deprivation involves retrograde pathway tracing. This method employs anatomical tracers (typically dextrans), weighing 3,000 daltons, which are injected into a region of interest. The tracer is taken up by axon terminals, and retrogradely transported to label all cortical and thalamic cell bodies projecting to the injected area. Several auditory areas have been examined with this method, such as those previously discussed – A1, A2, DZ, and PAF (Chabot et al., 2015; Butler et al., 2018; Kok et al., 2014; Butler et al., 2016). It had been hypothesized that areas known to cross-modally reorganize may receive increased input (resulting in greater numbers of projecting cells) from bimodal, thalamic, or non-auditory areas. Reflecting increases to A2 volume, retrograde injections into A1 revealed an increased projection from A2 in deaf cats compared to hearing cats (Chabot et al., 2015). Therefore, an explanation for the increase in A2 volume could be due to an increase in the number of neurons in A2. Chabot and colleagues additionally found no change in the projection from other cortical or thalamic areas to A1. This is in accordance with previous research indicating a lack of cross-modal activity in A1 (Kral et al., 2003).

Similar studies of afferent projections to PAF and fAES, areas known to reorganize cross-modally, have not shown any difference in the number of neurons projecting from other cortical and thalamic areas. However, the higher order area DZ showed some small increases in the projection from visual areas (Kok et al., 2014). It was found that DZ receives an increased projection from neighbouring posterolateral lateral suprasylvian area (PLLS). This result indicates an increase in connection strength between visual cortex and deaf auditory cortex, to a cross-modally reorganized area.

Although there exists some anatomical evidence for cross-modal reorganization in deafness, the vast majority of research has revealed little change in the number of cells projecting to individual auditory areas. Recently, however, it has been proposed that the synapse is the level at which reorganization can be observed in auditory cortex. As previously discussed, one cat auditory area known to cross-modally reorganize due to deafness is the fAES (Meredith et al., 2011). It has recently been identified that dendritic spine density is significantly increased in the supragranular layers of fAES in deaf cats (Clemo et al., 2016). In light of a complete lack of change in distant cell body projections to fAES, it was hypothesized that dendritic spine increases would be accompanied by increases in axon terminal density, carrying non-auditory input (Meredith et al., 2016). These terminals could arise from corticocortical connections as well as altered thalamocortical input (Butler et al., 2017).

1.7 The Thalamocortical Perspective

The thalamus is often described as a processing and relay centre of sensory input to the cortex. However, the thalamus is also a vital forebrain structure which guides cortical development (O'Leary and Nakagawa, 2002). As discussed, the thalamus

typically receives sensory input from a neural receptor nucleus, such as the inferior colliculus. The medial geniculate body (MGB), is the thalamic nucleus which receives auditory input, and sends efferents to the auditory cortex. In combination with genetic influences (such as transcription factors), thalamocortical input and sensory input (or lack thereof) define much of cortical differentiation and specification (O'Leary et al., 2007).

Some transcription factors involved in cortical development are present before the arrival of thalamic input, but are differentially expressed after thalamic innervation of cortex. This points to a link between sensory input and genetic influences in prenatal cortical development (Nakagawa et al., 1999). It has been proposed that genetic influences on the differentiation between cortical areas require some level of thalamocortical input to be initiated (Nakagawa and O'Leary, 2003). Taken together with knockout studies of transgenic mice, it can be surmised that genes expressed in the developing cortex increase expression of transcription factors and attractive guidance molecules, such as ephrins, for thalamocortical input, which further promotes cortical differentiation (Hamasaki et al., 2004; O'Leary and Nakagawa, 2002). Therefore, from the developmental perspective, thalamocortical input plays a major role in the characterization of different cortical areas.

It is equally important to note the timing of the thalamic invasion of cortex, as it may pertain to the timing of sensory deprivation during development. In cats, thalamic invasion of cortex begins prenatally, and extends into the first week after birth. These thalamocortical axons then wait to make contact with migrating layer IV cortical neurons (Shatz and Luskin, 1986; Rakic, 1977). These findings, together with the notion of the

thalamus as the major source of sensory input to the cortex, make the thalamus a subject of interest for study in cross-modal plasticity.

1.8 Sensory Deprivation Modifies Neural Anatomy

Although there is a lack of anatomical evidence for cross-modal reorganization in the cortex, there has been extensive research on subcortical reorganization due to blindness and deafness. Similar to compensatory plasticity in deafness, blind individuals experience cross-modal reorganization, and enhanced auditory capabilities (Rauschecker, 1995). There is evidence for auditory activation of visual cortex in postnatally blind mice (Chabot et al., 2007). In a congenitally blind mouse model, a direct connection has been found between the inferior colliculus and the lateral geniculate nucleus (Chabot et al., 2008). This reorganization is also found in visual brainstem structures. The superior colliculus has been investigated in visual deprivation models, and found to contain neurons responsive to auditory stimuli, enabling the enhanced sound localization attributed to blindness (Rauschecker and Korte, 1993; Rauschecker and Kniepert, 1994). These studies are vital to the understanding of compensatory plasticity, since thalamocortical axons had already invaded cortex. This indicates a change in modality in the sensory input conveyed to the cortex from the thalamus.

It is equally important to study the thalamus in the context of prenatal sensory deprivation. Following embryonic bilateral enucleation in monkeys, it has been found that the lateral geniculate nucleus (LGN) contains fewer neurons and is smaller in size than in normal sighted counterparts (Rakic et al., 1991). Conversely, in similar enucleation studies in the opossum, increased projections were found between the MGB and visual cortex, as well as between auditory areas and primary visual cortex, (Karlen et

al., 2006). In support of this, blind macaques exhibit increased striate projections to primary visual cortex, indicating the lack of visual thalamic input as a method for cross-modal reorganization (Magrou et al., 2018). These findings suggested a mechanism by which the blind visual cortex is able to process auditory information. It is possible that sensory deprivation promotes cross-modal thalamocortical and corticocortical connections.

In deafness, however, there is limited research on subcortical cross-modal reorganization. One study, conducted by Hunt and colleagues, revealed an increased retinal projection to the MGB in congenitally deaf mice, indicating a potential switch in modality of sensory information in the thalamocortical pathway (Hunt et al., 2005). This connection can also be induced experimentally, through ablation of the inferior colliculus. As in the research by Hunt and colleagues, an increased projection was observed between the retina and MGB (Angelucci et al., 1997). Manipulations have also been performed in the ferret, to redirect retinal efferents to the MGB. These manipulations have resulted in a visually responsive MGB, as well as a visually responsive A1, indicating that the deprivation-induced retinogeniculate connection is functional visually (Sur and Leamey, 2001). Taken together, these findings suggest a major thalamic role in the compensatory plasticity observed in the cortex, such that thalamic nuclei can carry altered sensory information (Mezzera and Lopez-Bendito, 2016).

1.9 Research Question and Hypothesis

The nervous system is capable of immense plasticity. This plasticity can be examined through models of sensory deprivation, through which cross-modal reorganization can occur. As discussed, cross-modal reorganization is the process by

which deprived areas of the brain (i.e. auditory cortex in deaf individuals) are able to process sensory input from the spared/remaining senses. Examples of this in humans include enhanced face processing in deaf individuals, with this advantage localized to regions of auditory cortex (Benetti et al., 2017). In animal models, the cat has been extensively studied, with deaf cats exhibiting specific visual advantages over normal hearing cats. These advantages have included visual localization and motion detection, localized to areas of deaf auditory cortex (Lomber et al., 2010). While there have been profound discoveries in cross-modal reorganization employing behavioural, electrophysiological, and imaging techniques, the anatomical basis for this reorganization is very poorly understood. There are multiple hypotheses regarding this reorganization. First, it has been proposed that deafness modifies the number of cortical and thalamic neurons projecting to reorganized areas of auditory cortex. A second hypothesis proposes that the number of neurons projecting to auditory areas is maintained, while the amount of axon branching on the neurons is modified in areas of reorganized auditory cortex. A third possibility exists that combines both hypotheses, such that modifications are found in both the number of neurons and amount of axon branching (Rauschecker, 1995; Butler et al., 2016; Clemo et al., 2016). Retrograde tracing studies, attempting to identify novel or altered cell body projections to reorganized auditory areas, have largely concluded that the thalamocortical and corticocortical projections are maintained in deafness. These studies have proposed numerous alternatives to explain the anatomical basis for cross-modal reorganization. Among these alternatives are that the level of reorganization is located at cortical synapses. Therefore, this thesis will examine the likelihood of the second hypothesis listed above.

Recent research has identified increases to dendritic spine density in deaf auditory cortex, indicating a possible complementary increase in axon terminals in regions known to cross-modally reorganize (Meredith et al., 2016). These increases in axon terminals could derive from thalamocortical or corticocortical connections. This project will address the hypothesis that the thalamocortical pathway (from MGB to cortex) is cross-modally reorganized, such that increases in axon terminal density are found in areas of "deaf" auditory cortex that have been identified to contribute to visual processing. There is some evidence that the MGB is reorganized due to sensory deprivation, such that the information it carries can switch modality, from auditory to visual (Sur and Leamery, 2001; Hunt et al., 2005). Taken together, it is possible that axon terminals in auditory cortex, originating in the MGB, are increased in density in certain areas. If the projection profile is maintained between hearing and deaf, the anatomical basis for cross-modal reorganization may be in corticocortical circuits. It is necessary to investigate these questions in order to examine the timing of critical periods in development. Within the field, questions remain about the physiological differences and degree of reorganization in congenitally deaf, early-deaf, and late-deaf individuals. This is pertinent to the study of cochlear implants, which appear to be less successful in subjects having undergone longer periods of sensory deprivation. Therefore, the current study attempts to provide an anatomical explanation for cross-modal reorganization in the deaf auditory cortex, with implications for cortical development. To examine thalamocortical projections, anterograde tracers were deposited in the MGB of hearing and deaf cats. This study demonstrates a conserved projection to auditory cortex, and an increased projection to

specific visual areas, indicating a change in the sensory information that is processed in the MGB.

Chapter 2

Methods

2.1 Experimental Subjects

A total of 8 adult domestic short-haired cats were examined to identify possible changes in thalamocortical axon terminals, as an anatomical basis for cross-modal reorganization induced by deafness. Each cat received bilateral injections of anterograde fluorescent tracers. Alexa Fluor 488 was deposited in the left MGB, and Alexa Fluor 594 was deposited in the right MGB. Cats were selected in this experiment due to a number of important factors. First and foremost, cats have long been a preferred animal model for studies on the auditory system due their relatively large brains, with similar auditory cortex to that of humans. In addition, the present study is largely concerned with the auditory thalamus. It has been found that cats exhibit similar cell types and intrathalamic cellular interactions as in humans (Sherman and Guillery, 2002).

One cat was obtained directly from a USDA licensed breeding facility (Liberty Laboratories, Waverly, NY). The other 7 cats were born in-house from pregnant queens obtained from Liberty Laboratories. Cats were housed in an enriching environment, with numerous toys and climbing structures. Food was provided for one hour each day and water was provided ad libitum. Cats were divided into two groups. Four cats comprised the normal hearing group, and four cats comprised the early-deaf group. Early-deaf cats were ototoxically deafened within the first 3 postnatal weeks, during the critical period of auditory development. All surgical and experimental procedures were completed in accordance with the Canadian Council on Animal Care's "Guide to the Care and Use of

Experimental Animals" (Olfert et al., 1993) and approved by the University of Western Ontario Animal Use Subcommittee of the University Council on Animal Care. The experimental timeline for each cat is depicted in figure 2. 1.

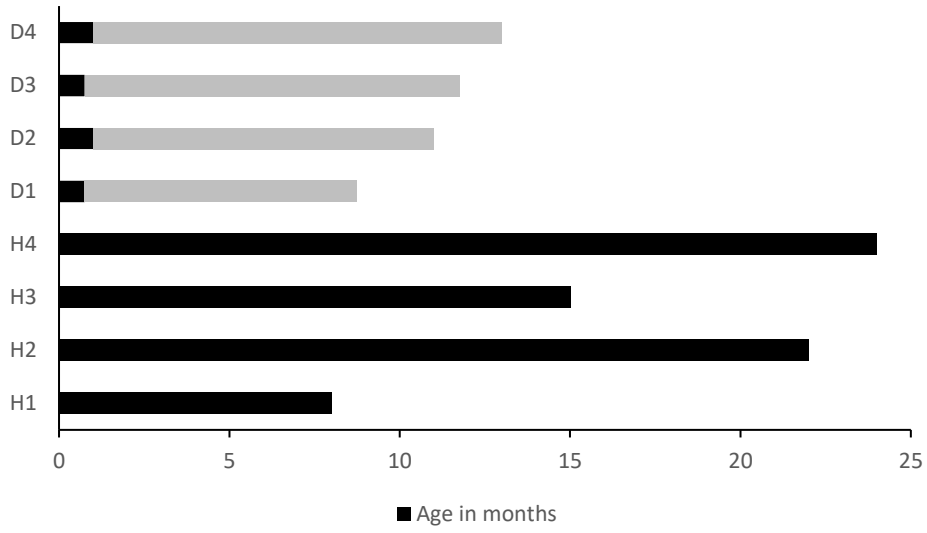

Figure 2. 1 Timeline of hearing and deafness for 8 cats examined in this study. The length of black bars represents duration of hearing. The length of grey bars represents the duration of deafness. The end of each bar represents tracer injections (of Alexa Fluor 488 and 594, deposited into the left and right MGB, respectively) and perfusion procedures. Perfusion was conducted on cats at a minimum age of 8 months. Early deaf cats are shown in D1-D4. Hearing cats are shown in H1-H4.

2.2 Induction of Deafness

Deafness was induced by daily neomycin (200 mg/kg – Valeant Pharmaceuticals, Laval, Quebec) subcutaneous injections for the first 3 weeks postnatal. Neomcyin is known to effectively destroy hair cells, which results in permanent and profound hearing loss (Xu et al., 1993). It is typically assumed that animal models of profound deafness require ABR thresholds of 80 dB across the frequencies tested (WHO, 2018). Over the same period of deafening, control cats (normal hearing) were administered daily saline injections, of the same volume administered to the deaf group.

2.3 Confirmation of Deafness

Deafness was confirmed by an absence of an auditory brainstem response (ABR) to acoustic stimuli at least 80 dB in intensity. The ABR was assessed at 3 weeks postnatal, as this is after the timing of hearing onset in the cat (Shipley et al., 1980). Auditory brainstem responses (ABR) are used to measure the extent and success of deafness in animal models. Wave I of the ABR is of particular interest, as its activity represents that of the auditory nerve. Through ABR measurements, profound deafness can be defined as lack of waves I to V at 80 dB (Starr, 1976). The ABR was conducted by placing subdermal EEG leads above the ears and on the vertex of the scalp (with a reference at the lower back) to record thresholds of hearing. Auditory stimuli (0.1-ms squarewave clicks; range 20-80dB nHL) were presented through ER3A foam insert headphones (Etymotic Research, Elk Grove Village, IL). Cats were anesthetized by isofluorane administration (5% to begin, 1.5%-2% for procedure) and oxygen inhalation (1 L/min). Once the ABR measurement was completed and the anesthesia was discontinued, the cat recovered, and was returned to housing. Follow up ABRs were

performed 3 months after initial testing, in order to ensure that complete and profound deafness had occurred. Cats were only included in the experiment if deafness was confirmed by an absence of responses (flat ABR) at all stimulus intensities presented.

2.4 Tracer Deposits and Surgical Procedures

Pathway tracers were deposited not less than 8 months of age in deaf animals, and not less than 8 months of age in hearing animals (Fig. 2. 1). Cats were fasted the day prior to surgery, and administered ketamine (4 mg/kg – i.m. – Pfizer Animal Health, Exton, PA). In order to administer the anesthetic regime during surgery, an indwelling feline catheter was placed into the cephalic vein of each cat. To ensure stability, and confirm the correct placement in the vein, each catheter was flushed with heparinized saline. Each cat was then given a dose of dexamethasone (anti-inflammatory - 0.05 mg/kg, i.m.), and placed in individual housing overnight. The next day, each cat was administered atropine (0.02 mg/kg s.c.) in order to reduce respiratory and alimentary secretions, acepromazine (0.02 mg/kg, s.c.), dexamethasone (0.5 mg/kg, i.v.), and buprenorphine (0.01 mg/kg, s.c.). General anesthesia was induced with sodium pentobarbital (25 mg/kg to effect, i.v.), delivered through the indwelling catheter. Cats were then intubated, after being given Cetecaine, a topical anesthetic, sprayed onto the pharyngeal walls, to supress the gag reflex.

To prepare for surgery, hair was removed from the dorsal surface of the cat's head by shaving and application of Nair™ depilatory cream. Following hair removal, the cat's head was secured in a stereotaxic apparatus. Antiseptic protocols were then undertaken to prepare the cat for surgery. Several vital measurements were recorded throughout the experiment, including respiration rate, core temperature, blood pressure, and heart rate. A

water-filled heating pad (Gaymar, Orchard Park, NY) was placed beneath the cat, in order to maintain body temperature at 37°C. A midline scalp incision was made, and the left temporalis muscle was reflected laterally. A craniotomy was made from AP 0-10 and L 5-15 (Fig. 2. 2). The skull piece was removed and the dura reflected laterally. Alexa Fluor 488 Dextran (10,000 MW, [10% in saline], Invitrogen, Carlsbad, CA, Cat# D22910) was pressure-injected (1µL Hamilton 7001KH Syringe, Hamilton Company, Reno, NV). The 10K dextran conjugates have been used as reliable neuronal tracers, labelling anterograde pathways. The attached fluorophore on these dextrans allows identification of labelling by fluorescent microscopy (Reiner et al., 2000). Fluorescent dextrans have been shown to be reliable and sensitive neuroanatomical anterograde tracers, with more robust outcomes than other fluorophore-based tracers, in part due to their ability to be fixed with aldehydes (Nance and Burns, 1990).

Due to the subcortical nature of the MGB, injection penetrations were made through the suprasylvian gyrus of the visual cortex, in order to access the thalamus. Injections of 80nL were made at 2 depths, to cover the full dorso-ventral extent of the MGB. The first injection was made at the deeper (more ventral) aspect of the penetration, approximately 9mm dorsal to the base of the brain, using stereotaxic coordinates L 9.5 and DV 10.5 (Reinoso-Suarez, 1961) . Three deposits were made at this depth. Following the last of these deposits, the syringe was elevated 1mm, and the second injection (3 deposits) was commenced. Each injection was made over 12 minutes. The deeper injection was performed first in order to prevent tracer travel up the path of least resistance (ie. up the length of the syringe). Once all deposits were completed (3 per

depth), the exposed brain was photographed (Fig. 2. 2). The craniotomy was sealed with dental acrylic, which was anchored to the surrounding skull by stainless steel screws.

The right hemisphere injection into the MGB utilized Alexa Fluor 594 Dextran (10,000 MW, [10% in saline], Invitrogen, Carlsbad, CA, Cat# D22913). As with the left hemisphere injection, the syringe penetrated the suprasylvian gyrus, and the same depths were targeted for tracer deposits. When the final right hemisphere injection was completed, the syringe was slowly removed, and the craniotomy sealed identically to that of the left hemisphere. Once the acrylic hardened, the sodium pentobarbital regime was discontinued, and replaced with isoflurane (1.5%). The margins of the incision were injected with lidocaine subcutaneously, followed by sutures.

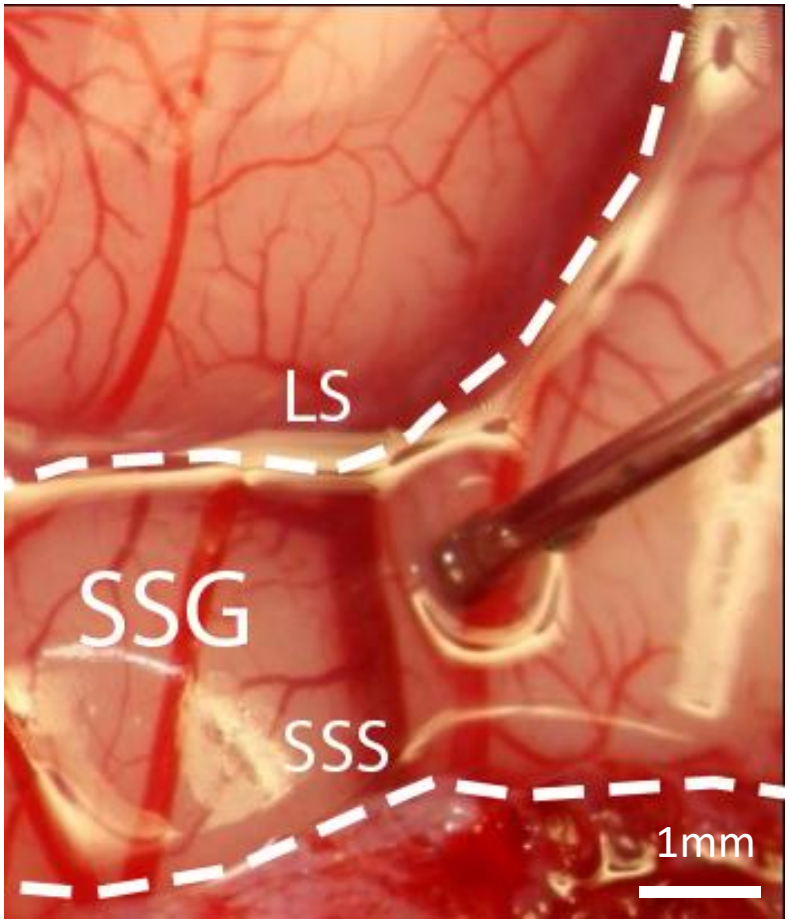

Figure 2. 2 Injection location of syringe through the suprasylvian gyrus, targeting the subcortical MGB.

Alexa Fluor 488 was pressure injected via a Hamilton syringe through the suprasylvian gyrus (SSG). Lateral sulcus (LS), suprasylvian sulcus (SSS). Anterior to the left, medial to the top.

2.5 Postsurgical Procedures

Upon observation of a swallowing reflex, the cat was extubated. For recovery, lactated Ringer's solution was administered subcutaneously as necessary. The vital signs mentioned previously (respiration rate, core temperature, blood pressure, and heart rate) were measured until the cat reached sternal recumbency. At this point, the indwelling catheter was removed, and the cat was returned to individual housing, with continuous monitoring. Buprenorphine (0.01 mg/kg, s.c.) was administered every 6 hours for the first 24 hours postoperative (marked as the time of extubation), and every 12 hours for the subsequent 72 hours. Each cat was also provided daily dexamethasone injections (s.c. 0.05 mg/kg on day 1, decreased by 0.01 mg/kg each day thereafter) for 5 days following the surgery. Between the 5^{th}, 6^{th}, and 7^{th} days following surgery, the volume of dexamethasone administered was decreased by half. In all cases, recovery was uneventful.

2.6 Perfusion and Tissue Processing

Two weeks after pathway tracer deposit, each animal was perfused with aldehyde fixatives. A catheter was placed into the cephalic vein, and cats were anesthetized with sodium pentobarbital (30mg/kg, i.v.). Heparin (anticoagulant - 10,000 U; 1 mL) and sodium nitrite (vasodilator - 1%, 1 mL) were administered (i.v.). Cats were then perfused intracardially through the ascending aorta with 1 liter of physiological saline, 2 liters of 4% paraformaldehyde (PAF - fixative solution), and 2 liters of 10% sucrose solution. The 10% sucrose initiated cryoprotection of the tissue. Each solution was buffered to a pH of 7.4 with 0.1 M Sorenson's buffer and infused at a rate of 100 ml/min. After the perfusion, the brain was exposed and blocked in the coronal plane at Horsley–Clarke level A27

(Horsley and Clarke, 1908). The brain and uppermost portion of the spinal cord were then removed and immersed in a 30% sucrose solution. After sinking, the brain was histologically processed to reveal the pathway tracer.

Brains were frozen and sectioned using a cryostat Leica CM 3050s (Leica Microsystems, Nussloch, Germany). Sections were collected at 60µm, in 6 series, such that 2 adjacent sections in the same series were separated by 360µm. The first series was directly used for fluorescent imaging, and quantification of axon terminals. The second series was immunohistochemically processed using the monoclonal antibody SMI-32 (Sternberger Monoclonals, Lutherville, MD, Cat# SMI-32, RRID:AB_2315331; Sternberger and Sternberger, 1983; Mellott et al., 2010). The third series was stained with cresyl violet in order to label Nissl bodies, which would aid in cortical and thalamic border delineation. The remaining series were saved as spares and used as necessary. All tissue was mounted onto gelatin-coated slides, air-dried, cleared, and coverslipped with a combination of graded alcohols, histoclear, and xylene mounting medium.

2.7 Data Analysis

Anterogradely labelled axons and axon terminals were visualized using a Nikon 90i microscope equipped with a Nikon C-HGFI Intensilight epi-fluorescence illuminator, and a QImaging EXI digital camera. Contour traces of tissue sections, and labelled axon terminals were plotted through Stereo Investigator® software, using a motorized stage controller (MBF Bioscience, Williston, VT). The series stained for SMI-32 reactivity allowed for delineation of individual cortical areas, and was viewed alongside the fluorescent sections, in order to draw contour traces around each area of interest. SMI-32

staining labels neurofilament protein subunits of pyramidal cells and dendrites in a characteristic manner across cortical areas (Mellott et al., 2010, see Fig. 2. 3). Within each area, an unbiased sampling method was used, aiming for confidence estimates at or below 0.1, indicating high levels of accuracy for any given estimate. Axon terminals, identified as swellings along the length or at the end of axons, were counted within each counting frame (Fig. 2. 3). Counting frames were 100x100µm. Raw counts of axon terminal labelling within each counting frame were extrapolated to the entire selected area (ie. the entirety of DZ in that section). The entirety of the z-plane of each section was scanned, with 5µm guard zones at the top and bottom, to reduce the possibility of double counting. The sampling area was randomly assigned to each selected area (Kawagishi et al., 2014). It has been shown that the stability of dextran labelling is unrelated to the age of the animal, and differences can not be simply attributed to age at injection/perfusion (Rajakumar et al., 1993).

Somatosensory and auditory cortical borders are marked by increases in SMI-32 reactivity (van der Gucht et al., 2001). These borders were confirmed by an absence of axon terminal labelling in somatosensory areas adjacent to auditory cortex. The borders between the posterior lateral suprasylvian areas (PLLS and PMLS), and the dorsal and ventral lateral suprasylvian areas (DLS and VLS) were identified as on the lateral bank of the middle suprasylvian sulcus and the anterior bank of the posterior limb of the suprasylvian sulcus, respectively (Palmer et al., 1978, Rauschecker et al., 1987). Axon terminals lying on the border between two areas were equally assigned to both areas. In both experimental groups, the relative proportion of thalamocortical projection to a cortical area was calculated by dividing the total estimated number of axon terminals in

that area by the total number of estimated labelled thalamocortical axon terminals in that brain. Relative proportions are preferred over raw terminal counts for meaningful analysis of projection strength, in order to account for possible variability in the uptake and spread of the tracer within the MGB. Labeling profiles were constructed for the deaf and hearing groups, and compared for differences in thalamocortical projection strength. An analysis of variance was conducted with post hoc Holm-Sidak corrections for multiple comparisons to determine connectivity between the MGB and individual cortical areas.

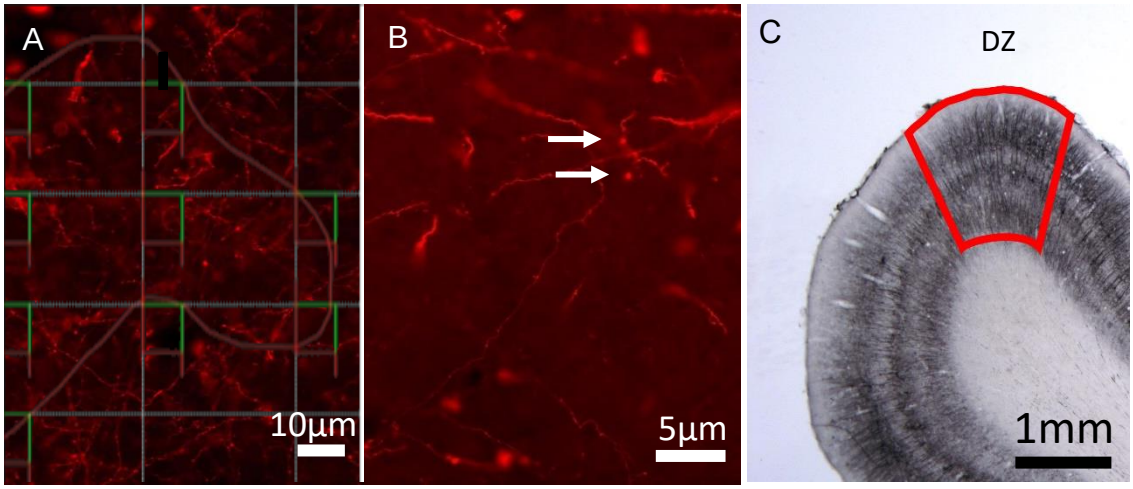

Figure 2. 3 Tissue samples showing the sampling method.

a) Tissue was sampled using Stereo Investigator® software (MBF Bioscience – Williston, VT), such that an unbiased estimate of axon terminal density in each cortical area could be made. b) Sample section of anterior auditory field, indicating labelling of axons and axon terminals with Alexa Fluor 594 anterograde tracer. Arrows indicate clear examples of axon terminals, defined as swellings along or at the end of axons. c) Parcellation of cortical area DZ via SMI-32 reactivity. Grid in a) not to scale.

Chapter 3

Results

3.1 Confirmation of Deafness

ABR protocols were used to confirm hearing or deafness in each subject (See section 2.2 for methodology). Examples are shown in Figure 3. 1. Hearing animals responded to sound stimuli with increasing latency with decreasing intensity. Deaf animals lacked responses to sound stimuli across intensities.

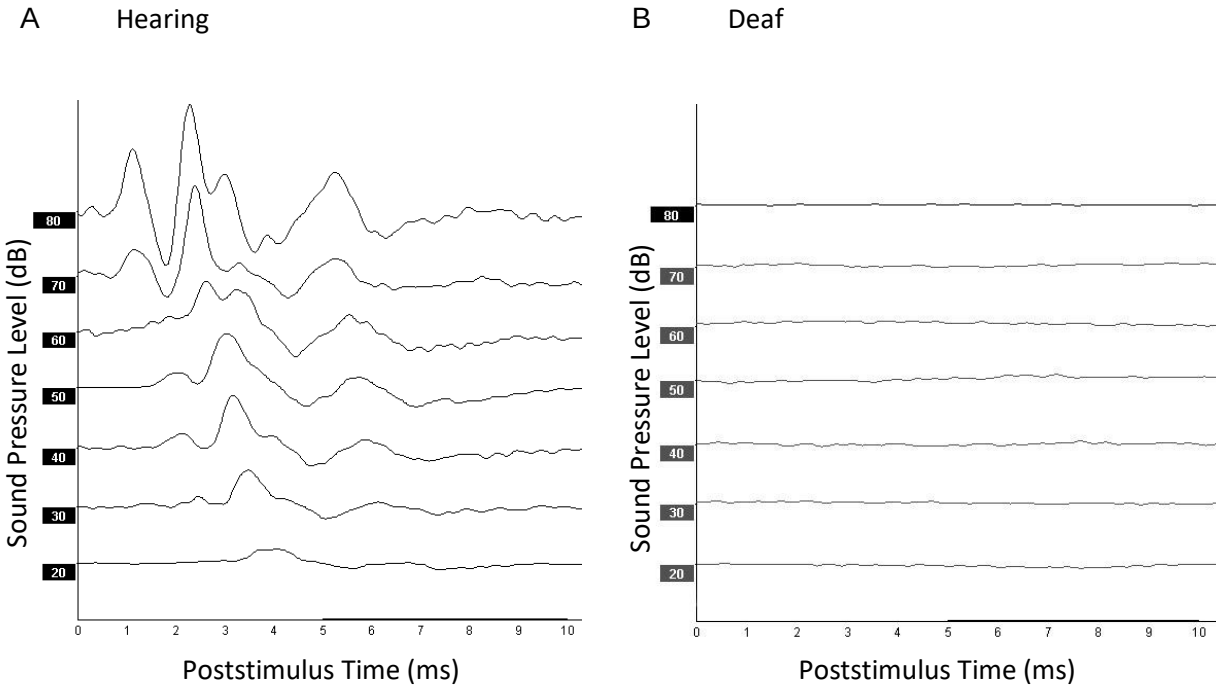

Figure 3. 1. Examples of auditory brainstem responses (ABRs) to sound stimuli (20-80dB).

A) Representative ABR from a hearing animal revealing typical responses to sound stimuli (0.1-ms squarewave clicks; range 20-80dB nHL). Peak latency increased with decreasing stimuli intensity. ABRs were conducted following the 3 week period of saline injections. B) Representative ABR from a deaf animal revealing an absence of responsiveness to sound stimuli. ABRs were conducted following ototoxic deafening procedures. Follow-up ABRs on hearing and deaf animals strongly resembled those in A and B.

3.2 Injection Sites

Eight cats received bilateral injections of anterograde fluorescent dextran tracers (Fig. 3. 2). Alexa Fluor 488 was deposited into the left hemisphere of each cat. Alexa Fluor 594 was deposited into the right hemisphere of each cat. Injections were made in each hemisphere at 2 depths to target all layers and divisions of the medial geniculate body (MGB). Due to the subcortical location of the MGB, surgical injections could not be directly visualized until after histological processing. The use of bilateral injections proved extremely useful, increasing the probability of accurate targeting of the MGB. Some injections revealed tracer spread into neighbouring white matter, or thalamic nuclei. Some of this spread could be due to the removal of the injection syringe upwards, towards the dorsal surface of the brain. This dorsal movement brings the glass syringe near or through the lateral geniculate nucleus (LGN). Tracer on the outside of the syringe, or at the tip may have accidentally been deposited in these areas. In order to ensure no spread into neighbouring areas, such as the LGN, each brain was examined for abnormal projections in primary visual cortex (area 17). Eight (3 left hemisphere, 5 right hemisphere) of 16 tracer deposits are included in this study, as one injection per animal was deemed unusable. Exclusionary criteria were spread of tracer into the LGN and other thalamic nuclei or cortical areas. Data was therefore collected from 5 right hemisphere injections, and 3 left hemisphere injections. Four representative cases of successful deposits are shown in Figure 3. 2. Alexa Fluor 594 covers the three major subdivisions of the MGB – ventral, dorsal, and medial, and the epicentre of the injection is localized strictly to the MGB. As seen in Figure 3. 2.d), spread of the injection was limited to the MGB. The LGN is pictured in the top right, with no tracer present.

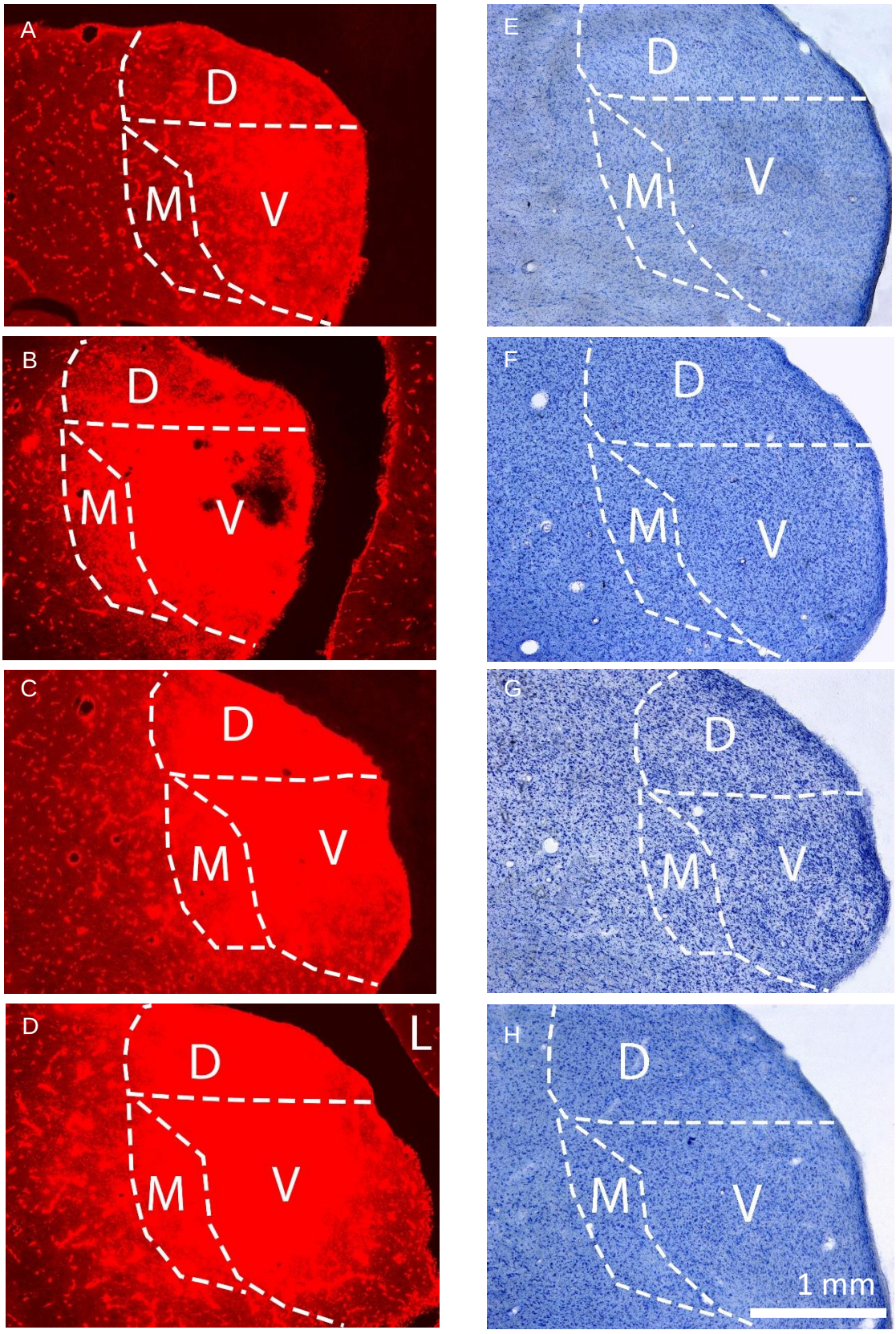
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Figure 3. 2 Injections of Alexa Fluor 594 in the right hemisphere of four cats.
a-d) Spread of the tracer injection within the thalamus. e-h) Nissl stained sections, directly adjacent to those in a-d. Injection spread is confined to the ventral (V), dorsal (D), and medial (M) divisions of MGB. Neighbouring areas, such as the LGN (L) in d), and white matter in b), reveal no labelling. Edges of the tissue appear brighter due to artifactual labelling, and unevenness. a,b) Injections in hearing cats H2 and H3, respectively. c,d) Injections in deaf cats D2 and D3, respectively. Top is dorsal, right is lateral. Fluorescent images b,d) are shifted laterally compared to f, h) in order to show adjacent tissue.

3.3 Summary of Axon Terminal Projection

3.3.1 Projection in hearing cats

The vast majority of axon terminal labelling was located in the hemisphere ipsilateral to the injection. Less than 0.001% of axon terminals were found in the contralateral hemisphere. Axons and axon terminals were consistently labelled in each of the 13 auditory cortical areas. In the hearing group, the greatest number of axon terminals were found in the core areas of primary auditory cortex (A1) and the anterior auditory field (AAF), and secondary areas including the second auditory cortex (A2), the posterior auditory field (PAF), and the dorsal zone (DZ). Substantial numbers of labelled axon terminals were also found in the remaining auditory cortical areas, namely the dorsoposterior ectosylvian gyrus (dPE), the intermediate division of the posterior ectosylvian auditory cortex (iPE), the ventral portion of the posterior ectosylvian auditory cortex (vPE), the auditory field of the anterior ectosylvian sulcus (fAES), the insular auditory cortex (IN), the temporal auditory cortical area (T), the ventral auditory field (VAF), and the ventral posterior auditory field (VPAF).

Labelled axon terminals were also identified in 14 areas of the visual cortex in the hearing group. Most of these visual projections were located in the anterolateral lateral suprasylvian area (ALLS), the anteromedial lateral suprasylvian area (AMLS), the posterolateral lateral suprasylvian area (PLLS), the posteromedial lateral suprasylvian area (PMLS), and the anterior ectosylvian visual area (AEV). These visual areas all share areal borders with auditory cortex, or are in close proximity to auditory cortex. MGB projections were also found in visual areas 17, 18, 19, and 7. Axon terminals were found

in much smaller quantities in areas 20a, 21a, 21b, as well as the ventral lateral suprasylvian area (VLS) and the posterior aspect of the posterior ectosylvian gyrus (EPp).

3.3.2 Projection in deaf cats

The projection to auditory areas in the deaf group greatly resembled that of the hearing group, with A1, AAF, A2, PAF, and DZ being the highest recipients of thalamic projections. Generally, increases in MGB axon terminals were found in dorsal areas of visual cortex, namely area 18 and PLLS of deaf cats. Overall, much of the projection profile to individual areas within auditory and visual cortex remained the same between hearing and deaf cats. Remarkably, the projection to visual areas increased in the deaf group, indicating reorganization of the thalamocortical pathway (Fig. 3. 3).

3.4 Projections by Modality

All labelled thalamocortical axon terminals in auditory and visual cortex were counted using Stereo Investigator® software (MBF Bioscience, Williston, VT). In order to generate the pie charts in Figure 3. 3, the total number of axon terminals in a modality (auditory or visual) were divided by the total number of axon terminals in that group (hearing or deaf). From these comparisons, it can be observed that there is a general increase in thalamocortical projection to visual cortex in deaf cats, accompanying a general decrease in thalamocortical projection to auditory cortex (Fig. 3. 3). Axon terminals found within auditory cortex decreased by 7%, and axon terminals found within visual cortex increased by 7% ($p=0.1846$, $t=1.408$). In summary, there is a modality-dependant change in thalamocortical crossmodal projection strength to auditory and visual cortical areas, which is caused by the onset of deafness.

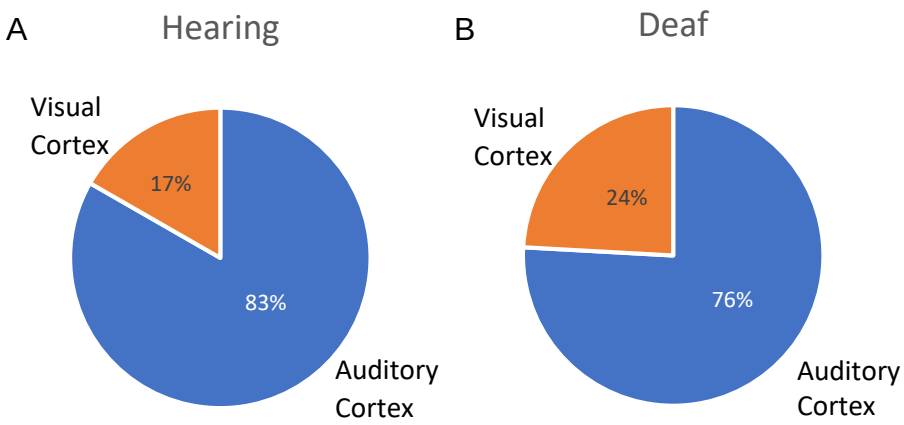

Figure 3. 3 Pie charts representing the total number of labelled axon terminals by sensory modality.

Axon terminals within a region are represented as relative percentages of all labelled axon terminals in that experimental group. a) Thalamocortical axon terminals in visual and auditory cortex in hearing cats. b) Thalamocortical axon terminals in visual and auditory cortex in deaf cats.

3.5 Differences in Projection Strength by Area

3.5.1 Ipsilateral Auditory Cortex

Differences in the thalamocortical connectivity between hearing and deaf cats were examined as a method for understanding the anatomical basis for crossmodal reorganization, and the enhanced visual abilities of deaf individuals. The density and distribution of axon terminals were examined throughout auditory and visual cortex. In auditory cortex, projections generally decreased in deaf cats compared to hearing cats (Figure 3. 3). Figure 3. 5 depicts comparisons between individual auditory areas in proportion of axon terminal labelling identified. An analysis of variance revealed an interaction between experimental group and the regions labelled ($F(26,162) = 1.677$, $p = 0.0285$). Axon terminal projections to primary auditory cortex (A1) were noticeably stable between hearing and deaf cats (hearing: 16.2%, deaf: 17.2% - $p > 0.99$, $t = 0.6083$). The other core auditory area, the anterior auditory field (AAF), exhibited a similar conservation in axon terminal labelling (hearing: 6.9%, deaf: 10.3% - $p = 0.75$, $t = 1.992$). Axon terminal labelling in secondary auditory areas, adjacent to A1 and AAF, was found to be stable. Specifically, projection strength to the posterior auditory field (PAF) remained largely stable between hearing and deaf cats (hearing: 12.1%, deaf: 9.3% - $p = 0.92$, $t = 1.63$). One secondary dorsal area, DZ, revealed another stable projection in the deaf group (hearing: 6.1%, deaf: 9.7% - $p = 0.66$, $t = 2.139$). More ventral auditory areas, such as area T, revealed similar stability in axon terminal labelling (hearing: 5.9%, deaf: 1.5%, - $p = 0.38$, $t = 2.583$ - see figure 3. 4 for example). Other ventral areas, such as vPE and VPAF, followed a similar trend as that of area T. The thalamocortical projection

strength to the remaining auditory areas was consistent between hearing and deaf cats, namely the fAES, an area known to crossmodally reorganize. The MGB pattern of projections to auditory cortex following deafness closely resembled that of hearing cats.

3.5.2 Ipsilateral Visual Cortex

In the visual cortex, axon terminal projections were found to increase in deaf cats (Figure 3. 6). Axon terminal density increased by 7% in visual cortex as a whole. MGB axon terminal projections to PLLS (hearing: 1.7%, deaf: 6.8%, see figure 3. 4 for example) were significantly greater in deaf than hearing cats ($p<0.0001$, $t=6.561$). MGB axon terminal projections to area 18 (hearing: 0.6%, deaf: 4.3%), were also significantly greater in deaf cats, compared to hearing cats ($p=0.0009$, $t=4.846$). The remaining labelling in visual cortical areas was consistent between hearing and deaf cats. Although the total projection strength to visual cortex was greater in the deaf condition, no individual area received more than 7% of the total thalamocortical axon terminal labelling.

3.5.3 Contralateral Cortex

Thalamocortical axon terminal projections to the contralateral hemisphere were minimal and rare, and are not shown.

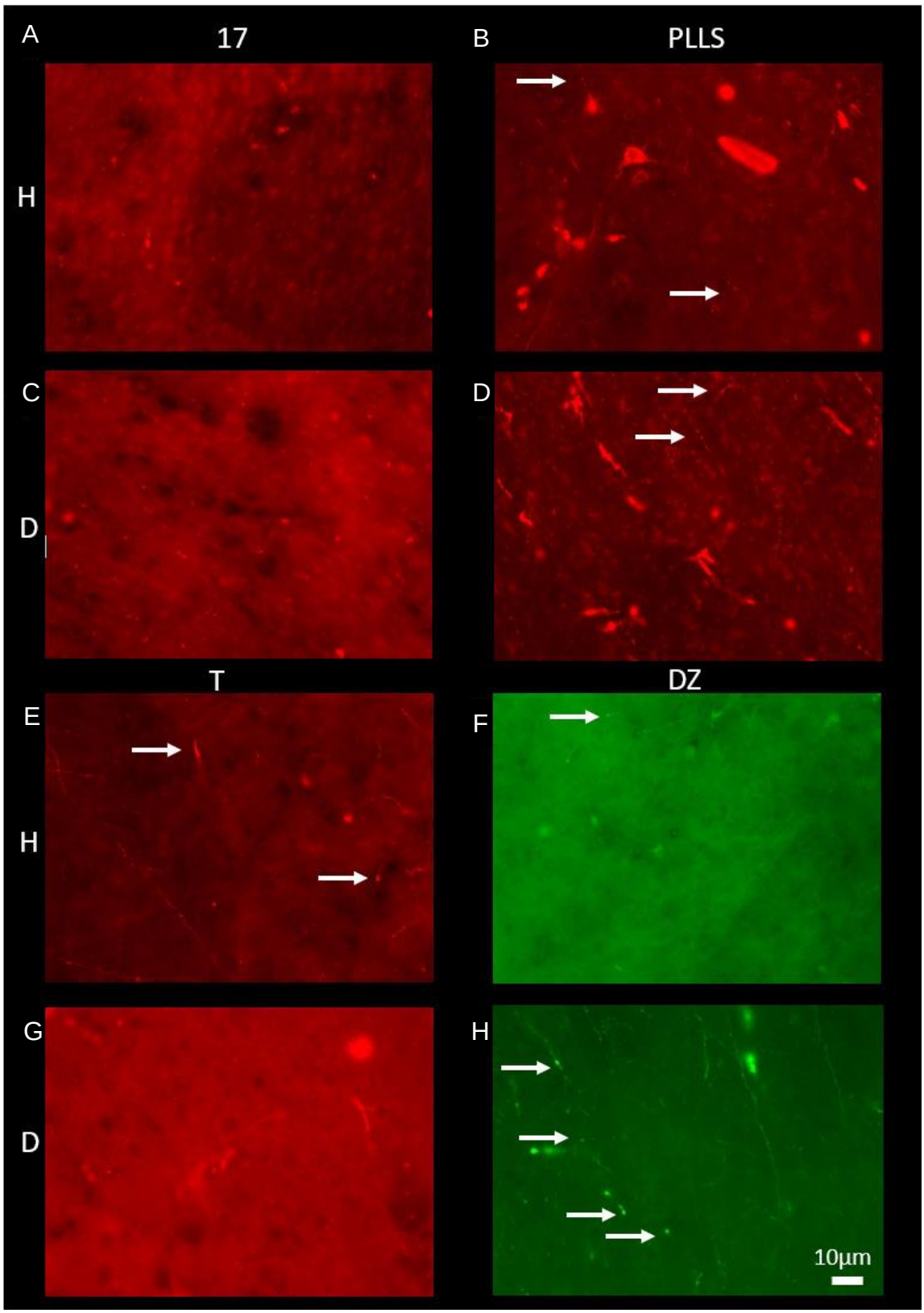
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Figure 3. 4 Illustrative comparisons of axon terminal density in specific cortical areas.

a,b) Axon terminal density in area 17 and PLLS, in hearing subjects (H). Note a distinct absence of terminals in area 17, with minimal labelling in PLLS. c,d) Axon terminal density in area 17 and PLLS, in deaf subjects (D). Deafness does not affect labelling in area 17, but does increase the projection to PLLS. e,f) Axon terminal density in area T and DZ in hearing subjects (H). Both areas exhibit moderate labelling. g,h) Axon terminal density in area T and DZ in deaf subjects (D). Labelling in both auditory areas is relatively stable between experimental groups. Arrows indicate clear examples of labelled axon terminals.

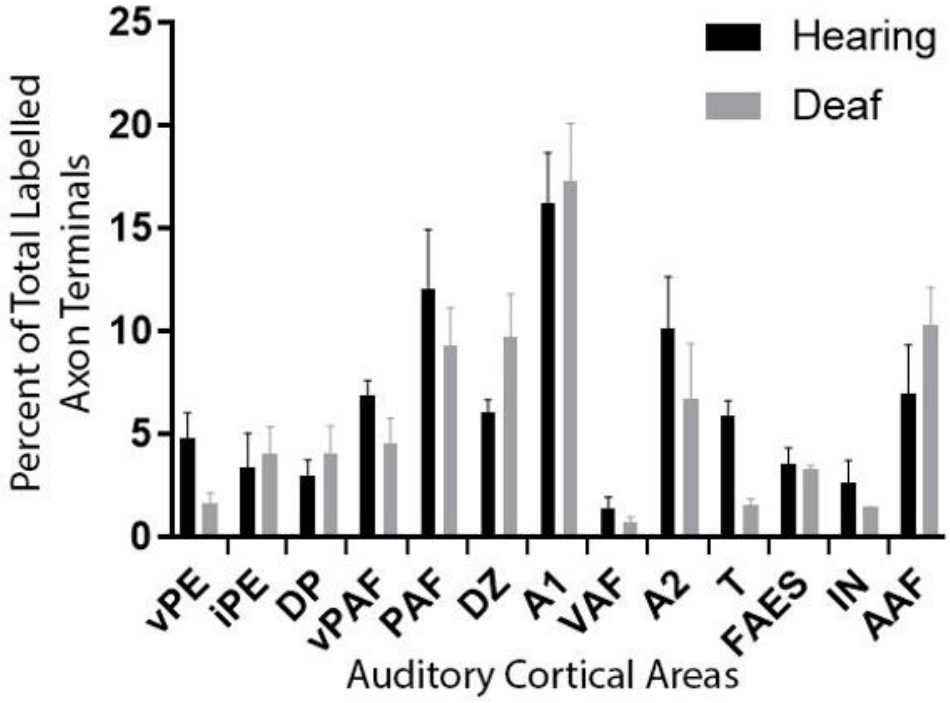

Figure 3. 5 Histogram depicting the distribution of thalamocortical axon terminals in the ipsilateral auditory cortex.

The y-axis represents the proportion of all labelled axon terminals in cortex. The x-axis represents individual auditory cortical areas in which labelling was observed. Error bars represent the standard error of the mean. Axon terminal distribution in auditory cortex is largely consistent between hearing and deaf cats. Cortical areas are ordered posterior (left) to anterior (right). For abbreviations, see list.

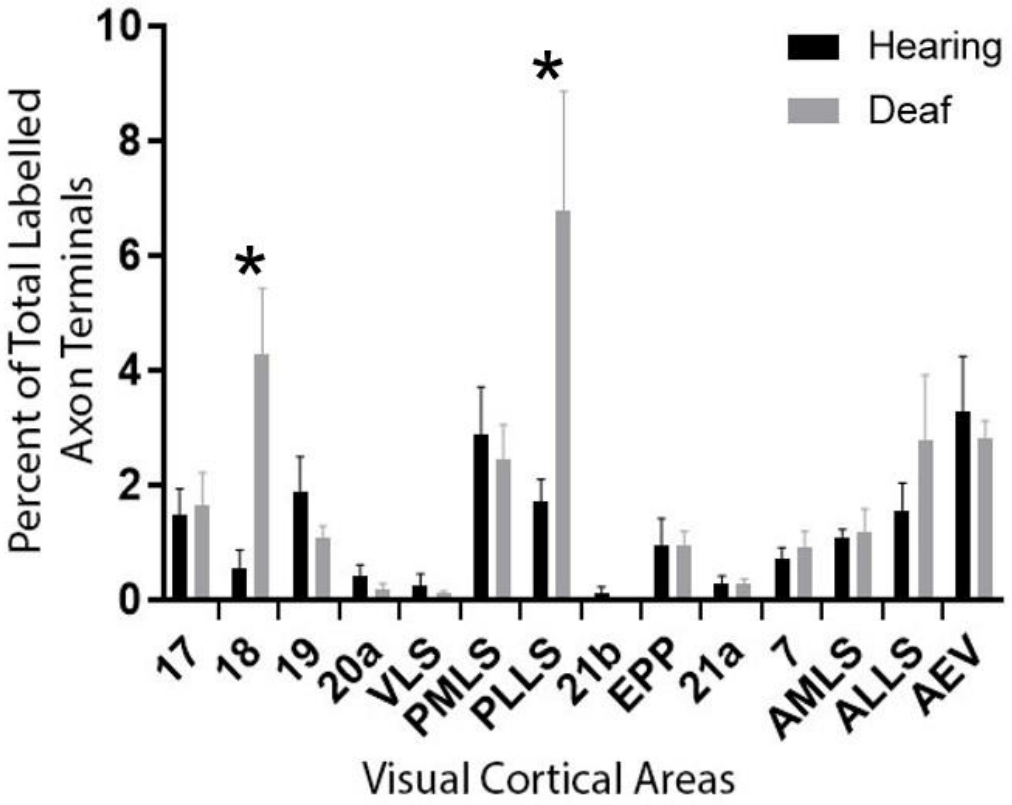

Figure 3. 6 Histogram depicting the distribution of thalamocortical axon terminals in the ipsilateral visual cortex.

The y-axis represents the proportion of all labelled axon terminals. The x-axis represents individual visual cortical areas in which axon terminal labelling was observed. Error bars represent the standard error of the mean. Asterisks represent significant differences between projection strengths ($P \leq 0.05$). Deafness results in increased projection strength from the MGB to visual cortex, with significant increases in the number of projections to PLLS and area 18. Cortical areas are ordered posterior (left) to anterior (right). For abbreviations, see list.

Chapter 4

Discussion

4.1 Summary

This study is the first to demonstrate the effect of deafness on thalamic axon terminal density in the cortex (MGB to cortex). It was found that axon terminal distribution is altered due to deafness, with areal-dependant modifications in MGB projection strength. A major finding of this experiment was that the projection to visual cortex greatly increased in the deaf group, while the projection to auditory cortex was conserved. Minor increases and decreases were observed in individual auditory areas. However, the mass stability of projections between hearing and deafness was apparent, even in functionally reorganized and bimodal areas such DZ, AAF, and area T. The remaining auditory areas appeared relatively unchanged in density and distribution of thalamocortical axon terminals. Of note, the PAF and fAES, areas known to crossmodally reorganize, did not exhibit any change in projection strength in the deaf group. Similarly, the majority of visual cortical areas were unchanged between hearing and deafness. However, two visual areas, PLLS and area 18, exhibited significant increases in projection strength in the deaf group. Extremely limited labelling of axon terminals was found in the contralateral hemisphere, and ipsilateral somatosensory cortex, and are thus not discussed.

Overall, this study provides anatomical evidence for crossmodal reorganization in deafness, with an emphasis on increased thalamocortical projection strength to visual cortex. Further, it appears that the connections between the MGB and the auditory cortex

are largely stable in deaf cats. These findings suggest a change in sensory information relayed by the thalamus to the cortex, supporting a hypothesis of increased axonal branching due to sensory deprivation.

4.2 Comparisons to Previous Research

The current study examined thalamocortical axon terminal projections in hearing and deaf adult cats. The labelling profile in hearing cats closely resembled previous studies of thalamocortical connectivity (Huang and Winer, 2000; Lee and Winer, 2008; Winer and Lee, 2007, see figure 4. 1). These studies made use of anterograde and retrograde pathway tracers to identify the full connectivity between the MGB and auditory cortex. In agreement with these investigations, the current study found the highest density of MGB axon terminals in A1, PAF, DZ, and A2, with smaller projections in the remainder of auditory cortex, namely VPAF, vPE, dPE, iPE, fAES, and T (Fig. 4. 1). It is important to note that the AAF, a core region of auditory cortex, receives comparatively less input from the MGB than other major auditory areas. This was confirmed in the present study, and is explained by the substantial AAF input from the rostral pole of the thalamus, rather than the MGB (Lee and Winer, 2008). The MGB was targeted as a whole in the present study, so as to examine axon terminal projections originating in the combined ventral, medial, and dorsal divisions. This injection strategy is relevant to the findings, as individual auditory areas receive a combination of thalamic inputs from the different MGB subdivisions (Morel and Imig, 1987).

The current study has attempted to expand on the existing literature surrounding thalamocortical connectivity, by investigating changes brought on by deafness. The most widely studied auditory area is the primary auditory cortex (A1). There has been

significant debate regarding the potential for A1 to crossmodally reorganize (Kral et al., 2003; Lambertz et al., 2005; Wong et al., 2014). Anatomical studies have been conducted, in order to define changes in numbers of corticocortical and thalamocortical neurons projecting to A1 (Stanton and Harrison, 2000; Barone et al., 2013; Chabot et al., 2015). Of these studies, only Chabot and colleagues found differences in neuronal projections from the MGB to A1. Specifically, deafness resulted in decreases in the ventral division, with similar sized increases in the dorsal division. It is possible that the increase in projection from the dorsal division compensated for the decrease in the ventral division, resulting in a more or less stable projection strength to A1, indicated by the stability of axon terminal density. In other terms, cell body counts, such as those reported, may not be directly indicative of axon terminal arborization in the cortex. However, a lack of evidence for large-scale changes in thalamocortical (total MGB) connectivity to A1 supports the finding that axon terminal density in A1 is unchanged by deafness. It is important to note that retrograde tracing studies have not yet found a definitive anatomical basis for crossmodal reorganization. Due to this controversy, a second approach was undertaken, involving counts of dendritic spines in auditory cortex. It was found that dendritic spines in the supragranular layers of deaf A1 increased in density (Clemo et al., 2017). Therefore, an accompanying increase in axon terminal density was expected. However, the thalamocortical projection to A1 was found to be stable between hearing and deaf cats.

The present study sought to define axon terminal distribution in an areal manner, rather than by cortical layer. It is possible that the supragranular thalamocortical input to A1 was increased in a similar manner to that of the dendritic spines. Therefore, it cannot

be concluded that thalamocortical axon terminal density in the superficial layers of A1 is not involved in crossmodal reorganization.

One auditory area, the dorsal zone, DZ, exhibited a conservation in axon terminal density in deaf cats. Unlike A1, it is well established that DZ reorganizes crossmodally, in order to better process visual motion in deaf cats (Lomber et al., 2010). Therefore, DZ has attracted significant attention and research into its anatomical pathways and connectivity. Unlike A1, which receives most of its thalamocortical input from the ventral division of the MGB, DZ receives a large portion of input from the dorsal division (Lee and Winer, 2008). However, the present study attempted to fill each MGB subdivision, allowing for more global labelling profiles to be constructed. In a number of studies, Kok and colleagues discovered a lack of thalamocortical and auditory corticocortical changes in connectivity to DZ (Kok et al., 2014; Kok and Lomber, 2017). Similar to findings in A1, it is possible that axon terminal distribution is completely separate from cell body counts at a distant nucleus. Indeed, it has been proposed that thalamocortical axon terminal weighting and cell body weighting have vastly different functions in the realm of auditory processing (Sherman and Guillery, 1996; Lee and Winer, 2008).

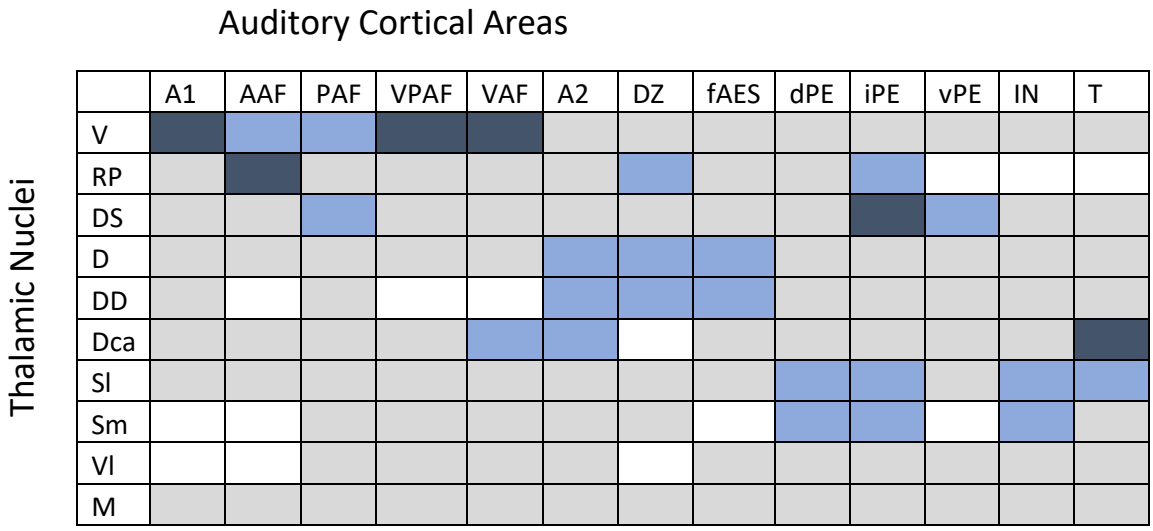

Figure 4. 1 Ascending (axon terminal) projections from various thalamic nuclei and subnuclei to the auditory cortex of hearing cats.

Darker rectangles indicate heavier densities of labelling. White rectangles indicate areas of zero labelling. A1, AAF, PAF, A2, and DZ appear to be among the most densely labelled recipients of axon terminals originating in the MGB. Auditory cortical areas are along the horizontal axis. Thalamic nuclei are along the vertical axis. MGB subnuclei are V (ventral), D (dorsal), and M (medial). Adapted from Winer et al., 2007.

Another auditory area, the posterior auditory field, PAF, is well known to reorganize crossmodally, and was considered a likely candidate to exhibit changes in axon terminal density (Lomber et al., 2010; Brown and Lomber, 2012; Butler et al., 2016). The present study found no change in thalamocortical projection strength to PAF. This is in accord with retrograde tracing studies, identifying thalamic cell bodies projecting to PAF (Butler et al., 2016). Taken together, there is not yet any anatomical evidence for crossmodal reorganization in PAF, as it appears that the thalamocortical pathway is stable between hearing and deaf cats. It is possible that anatomical reorganization in PAF is similar to that in A1, and may be dependant on cortical layer, as seen in supragranular-specific increases in A1 dendritic spines.

As discussed in the present study, virtually all labelled axon terminals were found in the hemisphere ipsilateral to the injection site. This finding indicates minimal or lack of contralateral hemispheric projections from the thalamus to the cortex. In fact, it was identified that all MGB originating cell bodies projecting to A1 are located in the ipsilateral thalamus (Chabot et al., 2015). Similarly, it has been found that all cells in the MGB projecting to the PAF are found in the ipsilateral hemisphere (Butler et al., 2016). Although not all auditory areas have been examined for contralateral thalamic connectivity, the results of Chabot, Butler, and colleagues support the current finding of a lack of contralateral thalamocortical labelling (Chabot et al., 2015; Butler et al., 2016).

In summary, the thalamocortical pathway to auditory cortex has been well studied. This research has taken the form of cortical retrograde tracing, and thalamocortical anterograde tracing (Huang and Winer, 2000; Butler et al., 2016; Chabot et al., 2015; Meredith et al., 2016). The present study is the first to extend the study of the

MGB axon terminal projection to deaf cats, and to nonauditory cortical areas. The hearing profile of axon terminal labelling found in this study closely resembled that of previous research in cats. Figure 4. 2 depicts summaries of the MGB projection to cortex. It was identified that many auditory areas maintain their connectivity with MGB following deafness. Regarding the % change panel, it was identified that no auditory area differed by more than 4% of the total labelling between hearing and deaf groups (Fig. 4. 2) The current findings also mirror the literature on thalamic cell body counts projecting to auditory cortex. However, they do not reflect increases reported elsewhere in dendritic spine density in deaf auditory cortex. These reported increases may be accompanied by layer-specific changes in thalamocortical input, rather than whole area modifications, which do not account for differences between cortical layers. Further, disparities between thalamocortical axon terminal and cell body counts to and from specific auditory areas may be attributed to differences in function.

4.3 Nonauditory Thalamocortical Projections

While there is significant literature on the connectivity between the MGB and auditory cortex, very limited research exists surrounding specific auditory thalamic inputs to visual cortex. One recent study demonstrated largely conserved thalamocortical input to V1 of deaf gerbils, while also revealing a strong pruning of unused projections during early sensory experience (Henschke et al., 2018). This study, like the majority of anatomical tracing research, focused on retrograde labelling of the thalamocortical pathway.

The current study is the first to use anterograde tracing to label axon terminal projections (originating in the MGB) in visual cortex. Significant changes observed in

axon terminal density were confined to visual cortical areas. Labelled axon terminals were consistently observed in 14 visual areas in hearing cats, with the majority found in PMLS, PLLS, ALLS, and AEV. In hearing cats, no individual area received more than 4% of the total thalamocortical projection. The total projection amounted to 17% of all labelled axon terminals. Two visual areas experienced significant increases in projection strength in the deaf group. These areas were PLLS and area 18, both of which are involved in visual motion detection (Pasternak and Maunsell, 1992; Lomber et al., 1994; Robitaille et al., 2008). The increased thalamocortical projection strength to PLLS is supported by previous research in pathway tracing and neuronal plasticity.

It is well established that DZ is an auditory area which is involved in visual motion processing in deaf cats (Lomber et al., 2010). Retrograde tracing studies involving deaf DZ revealed a significantly increased input from the neighbouring PLLS (Kok, et al., 2014). In hearing animals, PLLS is known to receive some input from the dorsal division of the MGB (Raczkowski and Rosenquist, 1983), although this has yet to be quantified in deaf animals. Taken together with knowledge that there is strengthened connectivity between PLLS and DZ in deaf cats, that the DZ crossmodally reorganizes to process vision, and that DZ and PLLS are both involved in motion processing, it may be that the increase in axon terminal density links thalamic input to the cortex. It is possible that the auditory thalamus is relaying visual information to the cortex, thus increasing its projection to visual cortex, and maintaining connections to reorganized auditory areas. For example, the present study has found increases in thalamocortical projection strength to area 18, and PLLS, and a stable projection to DZ. Input to PLLS and DZ may be accompanied by visual processing in the MGB, such that area 18 receives a strengthened

projection. This increased weighting of axon terminals may contribute to enhanced visual motion processing in DZ. There is yet no direct evidence for this pathway, as corticocortical studies of axon terminal distribution have yet to be examined in deaf animals.

In addition, a general increase in thalamocortical axon terminals to visual cortical areas (18 and PLLS) was observed. This change in the weighting of axon terminals may underlie a mechanism through which the deaf brain processes visual stimuli. Using blindness in cats as a model, it has been proposed that extended lateral axonal branching to deprived areas of sensory cortex provides the tool necessary for enhanced processing of the remaining sensory modalities (Rauschecker and Korte, 1993; Darian-Smith and Gilbert, 1994). Therefore one possibility, respecting Hebbian mechanisms, through which necessary connections are promoted and unnecessary connections are pruned (Hebb, 1949), is that the enhanced visual capabilities of deaf individuals are based in lateral corticocortical connections. These connections, as evidenced in the present study, may originate in nearby cortical areas of spared senses, such that an increase in axon terminal arborization is observed in deprived areas of cortex. For example, using the current data, an increased thalamic projection exists to area 18 and PLLS. These two spared visual areas may in turn send increased input to regions of deaf auditory cortex. This hypothesis is supported by the increase in connectivity between DZ and PLLS (Kok et al., 2014; see figure 4. 2). However, further corticocortical research involving anterograde pathway tracers must be undertaken to fully understand the extent of axon terminal distribution in deaf auditory cortex. One candidate for study would be to fully examine the PLLS

connection to DZ, by injecting anterograde tracers in PLLS, and identifying potential increases to axon terminal density in DZ and other crossmodally reorganized areas.

In summary, the present study is the first to identify input from the MGB to the visual cortex. In both hearing and deaf cats, thalamocortical axon terminals were labelled consistently in visual cortex, with an emphasis on areas bordering auditory cortex, or those known to support multimodal sensory processing. As seen in Figure 4. 2, the greatest increases in axon terminal labelling are localized to the above areas. Increases in axon terminal density were found in two areas, PLLS and area 18, known to be involved in visual motion processing (Fig. 4. 2). When compared to previous studies in corticocortical connectivity, it can be postulated that MGB input to the cortex may have undertaken a switch in sensory modality in deaf cats, from auditory to visual. In order to confirm this hypothesis, corticocortical axon terminal distribution should be examined.

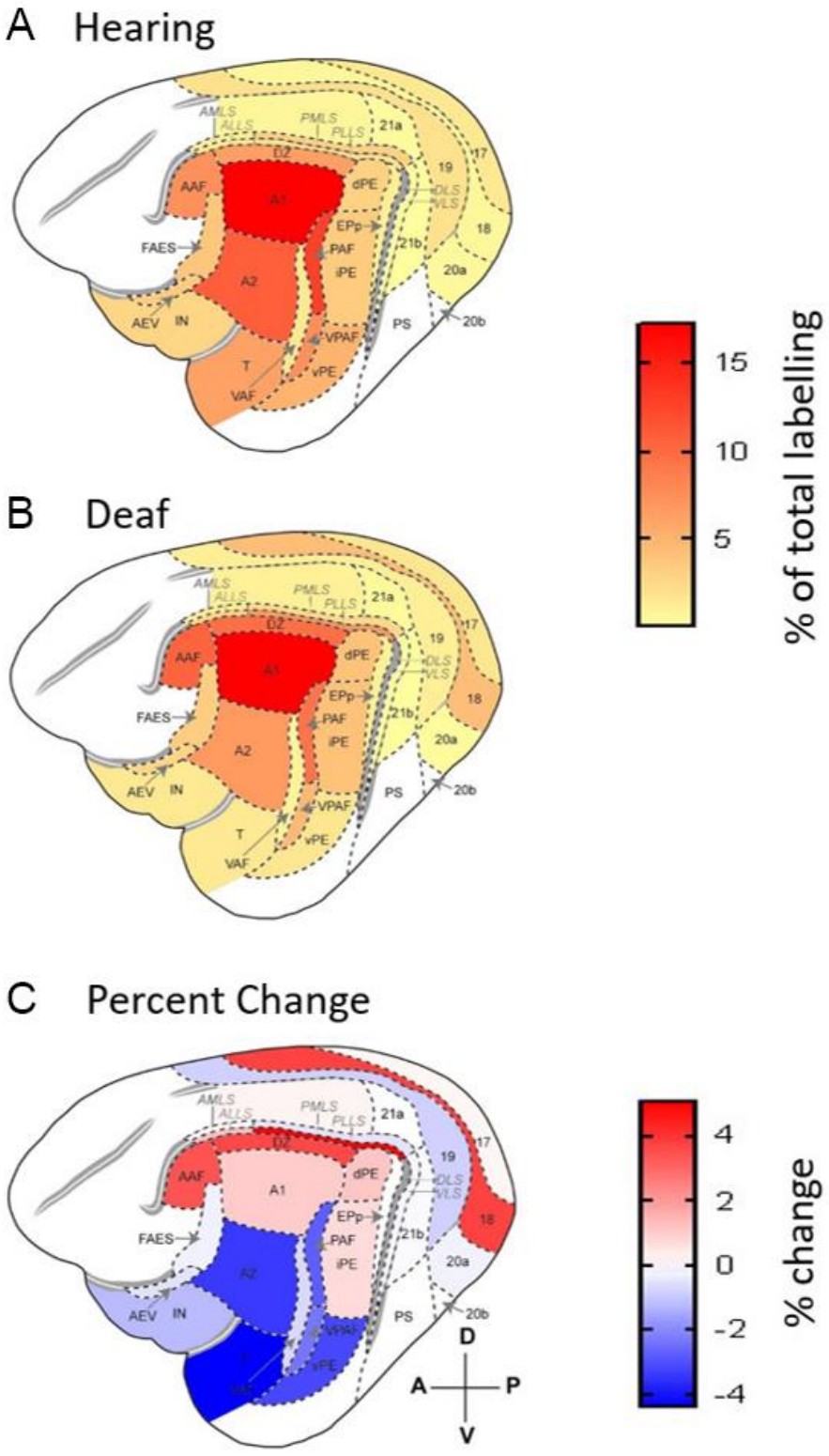
A Hearing
B Deaf
C Percent Change
AMLS
ALLS
PMLS
PLLS
21a
17
19
DLS
VLS
18
20a
20b
AAF
A1
dPE
EPp
PAF
iPE
21b
FAES
A2
AEV
IN
PS
VPAF
T
vPE
VAF
15
10
5
% of total labelling
4
2
0
-2
-4
% change
D
A
P
V

Figure 4. 2 Schematics of all axon terminal labelling by cortical area.

a) Summary of labelling in hearing cats. A central area including core auditory areas (A1 and AAF), and secondary areas (PAF, A2, DZ) are the major recipients of thalamocortical input. Projections to all visual areas are minimal. b) Summary of labelling in deaf cats. The major recipients of thalamocortical input are similar to those in hearing cats (A1, AAF, A2, DZ, PAF). Deafness reveals stable input to auditory areas, while increasing input to dorsal visual areas, or areas known to be involved in visual motion processing (PLLS and 18). c) Percent change of projections to cortical areas, as measured as the change observed in the deaf group (b) compared to the hearing group (a). *Increases* were found in dorsal visual areas or areas known to be involved in multimodal sensory processing (PLLS, AAF, 18)

4.4 Mechanisms of Change

As observed in the present study, thalamocortical input to the auditory cortex is largely conserved in deaf cats. This finding is consistent with previous literature involving individual retrograde pathway tracing studies in different areas of auditory cortex (Chabot et al., 2015; Butler et al., 2016; Kok et al., 2014; Meredith et al., 2016). It has been proposed that the conservation of projections to deaf auditory cortical areas allows for survival of existing connections, and supports potential restoration of normal hearing (Butler et al., 2016). This is in opposition to Hebbian pruning, whereby a lack of auditory stimuli prevents auditory synapses from exhibiting any correlated neural activity (Hebb, 1949). According to this theory, auditory synapses would be pruned away, and found to decrease in number in deprived areas. However, the existing literature points to a conservation of thalamocortical connectivity in deaf animals. At the surface level, this completely contradicts established Hebbian mechanisms. However, it appears that while feedback connections are subject to extended periods of plasticity, feed-forward connections are not (Batardiere et al., 2002; Price et al., 2006). The current study investigated the latter of these connections. It is therefore possible that axon terminal distribution in the cortex (originating in the MGB) is largely established before the onset of deafness, and is not affected by experience-dependant pruning mechanisms.

As discussed, the findings of this study indicate a conservation of thalamocortical projection strength to auditory cortex, with some statistically significant increases in axon terminal density in visual cortex. The question remains as to how the auditory thalamus is able to maintain connectivity with auditory cortex, and how deafness appears to promote axon terminal arborization in visual cortex.

In spite of a complete absence of auditory stimulation, the MGB maintains and even extends its connectivity to the cortex. The large-scale lack of change to thalamocortical axon terminal density and distribution is consistent with the literature on sensory development. As proposed elsewhere (Meredith et al., 2016), as an explanation for the absence of changes in connectivity observed in auditory cortex, the insult (onset of sensory deprivation) may be occurring too late in neural development to affect connectivity between cortical areas. In other words, the connections between cortical and thalamic areas may already be largely fixed by the time of hearing/deafness. This hypothesis is supported by research in development of the thalamocortical projections in the cat. By prenatal day E50, circuitry in this pathway has already begun establishing connections (Johnson and Casagrande, 1993; Hermann et al., 1994). If the onset of hearing is approximately 14 days postnatal in cats, it is possible that thalamocortical projections to the auditory cortex have become mostly fixed by the time of deafening (Meredith et al., 2016). This is supported by a lack of thalamocortical change in projection to auditory cortex which is able to reflect crossmodal reorganization in specific auditory areas (Butler et al., 2016; Kok and Lomber, 2017; Stanton and Harrison, 2000). This conclusion, taken together with the present findings, and an absence of thalamocortical axon terminal redistribution in auditory cortex, indicate a certain incapacity of the brain to reorganize anatomically in response to sensory deprivation. However, while thalamocortical projections to auditory cortex may be arrested before the onset of hearing, corticocortical projections only begin establishing after birth, and are further refined throughout postnatal development and hearing onset (Callaway and Katz, 1990). This finding agrees with DZ pathway tracing, in which a strengthened connection

to PLLS was observed in deaf cats (Kok et al., 2014). The current findings support this connectivity, with an increased MGB projection to PLLS, a visual cortical area. It is therefore possible that extraneous thalamocortical projections exist to non-auditory areas, and are pruned away in a Hebbian manner upon normal auditory experience (Hebb, 1949; Rauschecker and Korte, 1993). This theory is called neuronal exuberance (Innocenti and Price, 2005), and postulates that the developing brain forms vastly greater numbers of connections than those that survive into adulthood. This pruning is dependant on the nature of sensory experience, i.e. deafness, or normal auditory stimulation. Just as it has been thought that the absence of auditory input to the thalamus would promote pruning of thalamocortical connections, it is possible that sensory deprivation prevents pruning of extra-auditory thalamocortical projections (Chabot et al., 2015). This may be the cause for increases in axon terminal density in PLLS and area 18 of the visual cortex. It has been proposed that deprivation-induced maintenance of extraneous cross-modal projections occurs in combination with subcortical rerouting of sensory afferents (Hunt et al., 2006)

To summarize, there is a mass stability of axon terminal projections from the MGB to the auditory cortex between hearing and deaf cats. This conservation contradicts classic Hebbian theories on pruning, but is supported by literature on early-life neural development. Thalamocortical projections to auditory cortex appear to be fixed before the onset of hearing in cats, but corticocortical projections can still develop postnatally. This advances the need for anterograde pathway tracing studies in specific corticocortical connections. In addition, neuronal exuberance theories accept the potential for changes in non-auditory thalamocortical projections, such that deafness prevents normal pruning

mechanisms. These conclusions align with the current data, suggesting the reorganization of the MGB itself.

4.5 Visual Processing in the Deaf Auditory Pathway

The auditory cortex is well known to be highly plastic, allowing for adaptations to sensory deprivation. Deafness seems to induce visual processing in deaf auditory cortical areas, allowing them to switch modality in sensory processing, while maintaining functional specificity (Lomber et al., 2010; Meredith et al., 2011; Finney et al., 2001). The anatomical basis for this crossmodal reorganization is poorly understood. However, recent research points toward corticocortical connectivity (namely axon terminal distribution) as a mechanism by which the deaf auditory cortex can process visual stimuli (Clemo et al., 2016; Meredith et al., 2016; Kok et al., 2014). The current findings contribute to the existing literature, and provide evidence for increased thalamocortical input to specific visual cortical areas, which may be involved in the expanded visual activity of auditory cortex. The question remains how this visual activity of the auditory pathway begins. How is the thalamocortical (MGB to cortex) pathway able to transmit visual information to the cortex, and rewire to exhibit differential patterns of input to the cortex? And how is it possible that the thalamocortical input to auditory cortex is conserved in deafness?

Previous literature, often involving retrograde pathway tracing, has attempted to explain this conservation. In light of an absence of thalamic reorganization in deafness, it has been proposed that a more distant subcortical structure, such as the brainstem, is the site of anatomical reorganization in the deaf brain (Meredith and Allman, 2012). This

proposition is interesting, as it indicates the potential for subcortical and subthalamic deep brain reorganization due to sensory deprivation. Unfortunately, specific pathway tracing in subthalamic auditory brain structures has yet to be undertaken in deaf animals. One fascinating study made use of retrograde pathway tracers, in order to label all cell bodies projecting to the superior colliculus, a visual brainstem structure (Butler et al., 2018). It was found that the visual cortical projection to the superior colliculus decreased in deaf cats, while the auditory projection increased. It was concluded that deafness creates a dispersion in collicular connectivity with the cortex. However, Butler and colleagues did not examine thalamotectal projections, which may have also been altered by deafness. It is possible that the increases to auditory projection in the superior colliculus are mirrored in increases in cells projecting from the deaf MGB. This idea is supported by research in blindness, where the connection between the inferior colliculus and LGN is strengthened in anopthalmic mice (Asanuma and Stanfield, 1990). This finding would emphasize the potential for subthalamic crossmodal reorganization, and a visual processing in the auditory pathway following deafness. However, additional anatomical evidence of subthalamic reorganization due to deafness is highly limited.

One study in congenitally deaf mice has examined retinothalamic projecting axon terminals. It was found that deaf mice exhibit increases to retinal-originating axon terminal density in the ventral and dorsal divisions of the MGB (Hunt et al., 2005). This projection was not identified in normal hearing mice. This finding led to the conclusion that sensory deprivation induces reorganization at early levels of sensory systems, and has a cascade of effects throughout the cortex. Hunt and colleagues suggested that the absence of patterned auditory activity in the MGB promoted ingrowth of optic tract

axons, mediated by the presence of ephrin molecules (Hunt et al., 2005; Frisen et al., 1998). It was concluded that the modality of the thalamus is decided by its early development sensory input, as the MGB may be rewired for visual input due to deafness (Hunt et al., 2005). These conclusions lead to the assumption that retinal input to the MGB may be among the anatomical causes for deprivation-induced crossmodal reorganization. However, the functionality of these retinal inputs is less well understood. Alternatively, there is a significant literature devoted to rerouting of retinal afferents to auditory nuclei. Roe and colleagues examined visual responsiveness of ferret A1 after retinal efferents were induced into the auditory thalamus by lesioning the superior colliculus and specific visual cortical areas. It was found that A1 exhibited similar response properties to normal V1 (Roe et al., 1992). The retinal innervation of the MGB was imposed by removing normal targets of retinal input. Similarly, it has been found that the MGB is responsive to visual stimuli, after receiving retinal input, and providing visual information to auditory cortex (Sur et al., 1988; Pallas et al., 1994). Inducing retinal afferents to the MGB is a different method than those employed in the present study and other pathway tracing research. However, Sur and colleagues were able to mimic the natural thalamic innervation by the retina observed by Hunt and colleagues, and were able to test the higher order circuitry (thalamus, auditory cortex) for visual responsiveness. It is therefore plausible that the retinal innervation of the MGB provided the incentive for conservation of auditory thalamocortical projections in the present study, as well as an increase in projection to visual cortical areas. This pathway is depicted in figure 4. 3. Using DZ as an example, retinal input to MGB may allow conservation of thalamocortical projections, while promoting increased axon branching in

visual cortex. Together, visual input can be relayed to DZ from both auditory areas and visual areas (Fig. 4. 3).

To review, the anatomical basis for visual processing in the auditory pathway following deafness is poorly understood. In light of an absence of auditory thalamocortical reorganization, it has been proposed that crossmodal reorganization exists at a subthalamic level. Indeed, there is evidence for retinal innervation of the deaf MGB, and that this connectivity permits visual functionality in the auditory cortex. The effect of retinal input to the MGB may be twofold. First, it appears evident that the thalamocortical input to auditory cortex is switched in modality to process visual stimuli. It is possible that this switch, in combination with guidance molecules, allows conservation of thalamocortical circuitry. Second, the retinal input to the thalamus may promote non-auditory increases to axon terminal density in the visual cortex. These visual areas may aid the visual takeover of auditory cortex, by providing extra visual input, via promoted thalamocortical connectivity. As well, it is likely that visual processing in the MGB prevents normal Hebbian pruning of extraneous visual projections (see Figure 4. 3). These conclusions match the present study, in which thalamocortical input is conserved to auditory areas, and increased to visual areas.

4.6 Conclusions

The current study is the first to examine thalamocortical axon terminal density and distribution in the auditory and visual cortices, across hearing and deaf cats. Previous research has made use of retrograde tracers to identify cell bodies projecting to auditory cortex (Chabot et al., 2015; Kok et al., 2014; Butler et al., 2016; Meredith et al., 2016). These studies were conducted in light of the growing literature on crossmodal plasticity

of the auditory system (Lomber et al., 2010; Meredith et al., 2011; Finney et al., 2001; Benetti et al., 2017). However, the vast majority of results from the above pathway tracing studies revealed a mass conservation of projection patterns, including those between the MGB and the cortex. Recent research has provided evidence that instead of changes in cell body counts in other areas, changes at the level of the synapse within reorganized areas is a more likely candidate for the examination of plasticity (Clemo et al., 2016; 2017). Due to these conclusions, it was hypothesized that increases in auditory dendritic spines would be accompanied by increases to thalamocortical axon terminals. It was found, though, that axon terminal distribution in the auditory cortex was largely conserved between hearing and deaf cats. Interestingly, increases in projection were found to specific visual areas, PLLS and area 18, both involved in visual motion processing (Pasternak and Maunsell, 1992; Lomber et al., 1994). Specifically, PLLS is known to exhibit an increased projection to the deaf, reorganized DZ (Kok et al., 2014). These increased projections to visual cortex are possibly caused by visual processing in the deaf auditory pathway, such that retinal afferents innervate the MGB, and provide visual information to the cortex (Hunt et al., 2005; Sur et al., 1988). This input may prevent Hebbian pruning of extraneous visual thalamocortical input, as well as promote conservation of auditory thalamocortical patterning. Further, it appears that the expected increases in axon terminal density in auditory cortex were not observed. Taken together with the possibility of visual takeover of the auditory pathway, it is necessary to conduct corticocortical axon terminal studies, which may provide concrete evidence for reorganization of the auditory cortex. The present study provides more evidence for the timing of development of the auditory pathway, such that thalamocortical input begins to

fix before auditory experience (Hermann et al., 1994). These findings further emphasize the need for corticocortical research into crossmodal plasticity, as the irreversibility of some connections affect the success rate of cochlear implantation (Lee et al., 2001).

The exact critical period for crossmodal plasticity to occur, and the potential for a return to auditory experience following cochlear implantation in humans is still not well understood. Many individuals receive unsuccessful implants, in part due to the stability of reorganized cortex following deafness. The present research suggests that this stability is mirrored in the preservation of auditory thalamocortical projections between hearing and deaf cats. However, conclusions cannot yet be drawn about the exact timing of reorganization, and the complete mechanisms underlying it. The current study reveals the need for analyses of corticocortical connectivity, in order to fully understand the anatomical basis for crossmodal reorganization, and why cochlear implant outcomes remain highly variable.

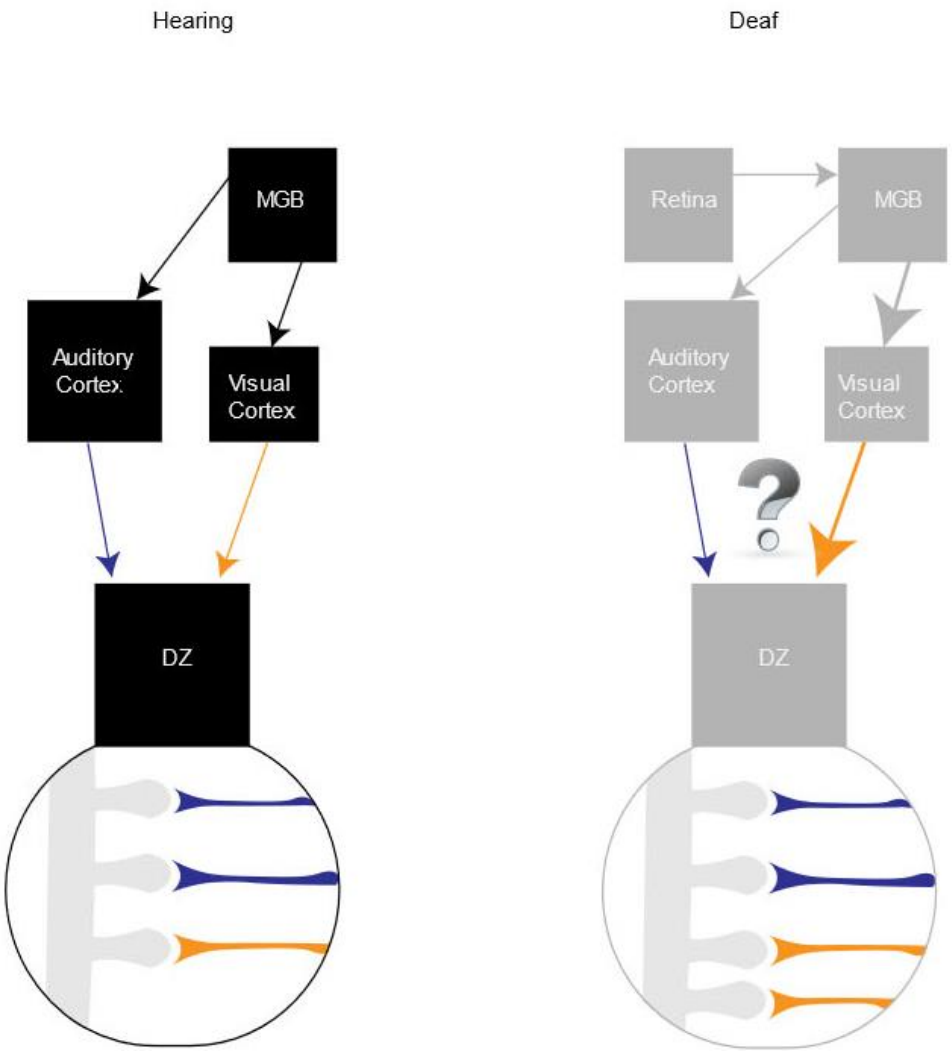

Figure 4. 3 Diagram of potential thalamocortical and corticocortical inputs to the DZ of auditory cortex.

On the left, the hearing DZ receives input from other auditory cortical areas, as well as thalamocortical input and non-auditory corticocortical input. On the right, deaf DZ receives visual input from other deaf auditory areas, as well as increased input from visual cortical areas. Adapted from Clemo et al., 2016.

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Appendix A

From: eSiriusWebServer <esiriusadmin@uwo.ca>
Sent: Friday, May 26, 2017 2:12 PM
To: Stephen Lomber
Cc: Animal Care Committee; esiriusadmin@uwo.ca
Subject: eSirius Notification - New Animal Use Protocol is APPROVED2017-013::1

Western

AUP Number: 2017-013
PI Name: Lomber, Stephen
AUP Title: Plasticity In Auditory Cortex
Approval Date: 05/26/2017
Official Notice of Animal Use Subcommittee (AUS) Approval: Your new Animal Use Protocol (AUP) entitled "Plasticity In Auditory Cortex" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal.2017-013::1

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.
Submitted by: Copeman, Laura
on behalf of the Animal Use Subcommittee
University Council on Animal Care

Curriculum Vitae

BENJAMIN TRACHTENBERG

EDUCATION

2016	B.Sc, Biomedical Sciences University of Ottawa, Ottawa, ON, Canada

RESEARCH EXPERIENCE

2014-2016	Undergraduate research assistant and honour's thesis student, Cerebro-Vascular Accidents and Behavioural Recovery Laboratory University of Ottawa, Ottawa, ON, Canada
2016-2018	Graduate Student, Cerebral Systems Lab University of Western Ontario, London, ON, Canada

HONOURS AND AWARDS

2012-2013	Admission scholarship, University of Ottawa
2016	Dean's List, University of Ottawa
2016-2018	Western University Graduate Research Scholarship

TEACHING EXPERIENCE

2016-2017	*Teaching Assistant* Physiology 3130z (UWO): Physiology Laboratory
2017-2018	*Teaching Assistant* Physiology 3000 (UWO): Physiology and Pharmacology Laboratory

CONFERENCE ABSTRACTS

Trachtenberg B, Butler BE, Lomber SG. *Modified Medial Geniculate Projections to Auditory and Visual Cortex Following Early-Onset Deafness*. (Conference Poster). International Multisensory Research Forum 2018, Toronto, Ontario, Canada