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THE EFFECTS OF LONG-TERM DEAFNESS ON DENSITY AND DIAMETER OF DENDRITIC SPINES ON PYRAMIDAL NEURONS IN THE DORSAL ZONE OF THE FELINE AUDITORY CORTEX

Rachel J. Bauer
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THE EFFECTS OF LONG-TERM DEAFNESS ON DENSITY AND DIAMETER OF DENDRITIC SPINES ON PYRAMIDAL NEURONS IN THE DORSAL ZONE OF THE FELINE AUDITORY CORTEX

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

By

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I would first like to thank my wonderful husband, Michael, who has remained patient and supportive through everything. I would also like to thank Dr. Meredith for allowing me to work with him on this project. He is clearly passionate and knowledgeable about science, but also about art, music, culture, and much more. I would like to extend that thanks to Dr. Clemo as well, who helped me many times with the program, interpretation, and more.
# Table of Contents

List of Figures ........................................................................................................iv
List of Tables .........................................................................................................v
Abstract ..................................................................................................................vi
Introduction ............................................................................................................1
Materials and Methods ..........................................................................................5
Results ...................................................................................................................10
Discussion .............................................................................................................31
References ............................................................................................................36
List of Figures

1. Location of the Auditory Dorsal Zone ......................................................... 4
2. Sample of Dendritic Segment .................................................................. 11
3. Density: Hearing vs Deaf ......................................................................... 14
4. Density: Hearing vs Deaf by Branch Order ............................................. 16
5. Density: Apical vs Basilar ...................................................................... 18
6. Density: Supragranular vs Infragranular .............................................. 20
7. Density: Apical vs Basilar in Supragranular and Infragranular ............. 21
8. Diameter: Hearing vs Deaf .................................................................... 24
9. Diameter: Hearing vs Deaf by Branch Order .......................................... 26
10. Diameter: Apical vs Basilar .................................................................. 28
11. Diameter: Supragranular vs Infragranular .......................................... 30
List of Tables

1. Summary of Spine Density by Laminar Position and Dendritic Type ………………13
2. Summary of Spine Density by Branch Order …………………………………………15
3. Summary of Spine Head Diameter by Laminar Position and Dendritic Type ……23
4. Summary of Spine Head Diameter by Branch Order ……………………………..25
Abstract

THE EFFECTS OF LONG-TERM DEAFNESS ON DENSITY AND DIAMETER OF DENDRITIC SPINES ON PYRAMIDAL NEURONS IN THE DORSAL ZONE OF THE FELINE AUDITORY CORTEX

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Major Director: A. Marvin Meredith, D. Phil.
Professor
Department of Anatomy and Neurobiology

Neuroplasticity has been researched in many different ways, from the growing neonatal brain to neural responses to trauma and injury. According to recent research, neuroplasticity is also prevalent in the ability of the brain to repurpose areas that are not of use, like in the case of a loss of a sense. Specifically, behavioral studies have shown that deaf humans (Bavalier and Neville, 2002) and cats have increased visual ability, and that different areas of the auditory cortex enhance specific kinds of sight. One such behavioral test demonstrated that the dorsal zone (DZ) of the auditory cortex enhances sensitivity to visual motion through cross-modal plasticity (Lomber et. al., 2010). Current research seeks to examine the anatomical structures responsible for these changes through analysis of excitatory neuron dendritic spine density and spine head diameter. This present study focuses on the examination of DZ neuron spine density, distribution, and size in deaf and hearing cats to corroborate the visual changes seen in behavioral studies. Using Golgi-stained tissue and light microscopy, our results showed a
decrease in overall spine density but slight increase in spine head diameter in deaf cats compared to hearing cats. These results, along with several other studies, support multiple theories on how cross-modal reorganization of the auditory cortex occurs after deafening.
Introduction

It has been well studied and established that in the absence of input of one sensory modality, the brain compensates by increasing the performance of one or more existing senses. This phenomenon is termed “cross-modal plasticity”. For example, when the brain is deprived of acoustic input, visual performance is supranormal. This has been demonstrated in studies on congenitally deaf and ototoxically deaf cats, as well as in humans, ferrets, and monkeys. In a previous behavioral study, deaf cats were found to have increased visual localization ability and increased visual motion detection (Lomber et. al., 2010). These changes transform the brain into a system that is functionally and structurally different from a normal hearing brain. Clinically, this could lead to a barrier to hearing restoration. For example, visually evoked activity in auditory cortex appears inversely correlated with speech perception scores in prelingually deaf children following cochlear implant (Buckley and Tobey, 2011). By better understanding how the brain reorganizes specific cortices after sensory loss, or cross-modal plasticity, we can better provide and predict clinical outcomes.

Currently, it is still not well understood how cross-modal plasticity occurs. While there is an increasing effort to investigate the neural bases for cross-modal plasticity, knowledge of the underlying brain circuitry remains virtually unknown. Recent studies of deafness-induced cross-modal plasticity in different subregions of auditory cortex indicate that the phenomenon is largely based on the “unmasking” of existing inputs. However, there is not a consensus on the sources or effects of cross-modal inputs to primary sensory cortical areas. Some studies have suggested that areas undergo complete reorganization, while others provide that cross-modal plasticity occurs only at selective regions (see review of Bavelier and Neville, 2002). Many investigations indicate that entire cortical areas vacated by the absent sensory modality are
completely replaced by inputs from the remaining systems (Bavelier and Neville, 2002). For example, imaging studies of cross-modal plasticity in early-deaf individuals have shown visual activation of auditory cortex partially including its primary levels (Finney et al., 2001; Lambertz et al., 2005), and Braille reading or tactile tasks activated visual cortices in blind subjects (Levänen and Hamdof, 2001; Sathian, 2000). These observations logically led to the assumption that all cortical areas possess the ability for cross-modal plasticity. The potential for such comprehensive reorganization is supported by results from studies using a series of neonatal lesions in animals (Roe et al., 1990; Sur et al., 1990). However, support for such global effects is not universal, and several studies (Nishimura et al., 1999; Weeks et al., 2000) specifically noted that primary auditory cortex was not cross-modally reorganized in their early-deaf subjects. These observations have also been substantiated by electrophysiological recordings from primary auditory cortices of congenitally deaf cats, which found no evidence of cross-modal plasticity (Kral et al., 2003). Therefore, when studying the neural basis for cross-modal plasticity, it is imperative to do so in a region where cross-modal plasticity has been documented.

Auditory cortical areas in which cross-modal plasticity has been demonstrated following deafness include the posterior auditory field (PAF), the dorsal zone (DZ), and the auditory field of the anterior ectosylvian sulcus (FAES) and the anterior auditory field (AAF) (Meredith & Lomber, 2011). In Lomber et. al., (2010), a behavioral study identifying areas responsible for supranormal vision after deafness, PAF was found to be responsible for peripheral vision localization, while DZ was found to enhance movement detection. In Meredith et al., (2011), FAES in deaf cats reorganized from acoustic orientation to visual orientation. Because these effects result in a change in response from auditory to visual activation, it is presumed that the basis for these changes occur at the excitatory synapse.
Dendritic spines serve as the post-synaptic basis for excitatory inputs to the principal cell type in cortex. Dendritic spine features, such as spine density and spine head diameter have been examined in several auditory cortical areas that demonstrate cross-modal plasticity after deafness. These studies have examined the primary auditory cortex (A1), and the auditory field of the anterior ectosylvian sulcus (FAES). These experiments showed an increase in spine density of supragranular neurons (Clemo et. al., 2014; Clemo et. al., 2017). Through these studies, we can better understand neuronal connectional configurations and how the brain responds to altered senses.

The present experiment was designed to further examine the mechanisms responsible for cross-modal plasticity, such as the ‘unmasking’ of existing but silent inputs and the introduction of novel inputs into the area. To do this, an area that is known to exhibit cross-modal plasticity, the dorsal zone (DZ), was selected. A diagram depicted DZ in relation to other auditory cortical regions of cats is depicted in Figure 1. Furthermore, DZ in early deaf cats is known to become cross-modally reorganized by visual inputs (Land et al., 2016). To determine if this effect is supported by changes in synaptic organization, Golgi-Cox staining and light microscopy techniques were used to compare dendritic spine density and spine head diameter in early-deaf cats when compared to their normal hearing controls.
Figure 1: A-D show various views of the location of DZ relative to other areas of the auditory cortex.
Materials and Methods

Cortical tissue from adult domestic cats involved in previous electrophysiological studies at the University of Western Ontario were used to comparatively analyze dendritic spine features in early deaf and hearing cats, as detailed below.

2.1 Animals and Ethics

All procedures for securing the tissue followed guidelines set by the National Research Council’s Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003), the Canadian council on Animal Care’s Guide to the Care and Use of Experimental Animals (Olfert et al., 1993), and with prior approval by the Institutional Animal Care and Use Committee at Virginia Commonwealth University or by the University of Western Ontario Animal Use Subcommittee of the University Council on Animal Care. Six unspayed female cats were used for this study (weight 2.9 – 4.8 kg and age 8.6 – 24.2 months; deaf average 16.2 months after deafening). The cats were at least eight months of age because the auditory cortex matures at ~six months (Kral et al., 2005). Male cats were not purposefully omitted.

2.2 Deafening and Tissue Preparation

Three hearing cats with normal auditory brainstem responses (ABR) served as the control group. The other three were ototoxically deafened prior to one month of age because the procedure is maximally effective after full hearing onset at ~15 days postnatal but before the auditory critical period that occurs at ~50 days postnatal (Xu et al., 1993). The ototoxic procedure was performed using a coadministration of one intravenous treatment of 60 mg/kg
sodium edecrin and one subcutaneous treatment of 300 mg/kg kanamycin (Xu et al., 1993). After treatment, deafness was confirmed by the absence of stimulus-evoked activity in the auditory brainstem response (ABR), as seen in other studies (Clemo et al., 2016; Allman et al., 2009; Kok et al., 2013; Wong et al., 2015). To euthanize, the adult cats were anesthetized with 40 mg/kg intravenous sodium pentobarbital, perfused with isotonic saline, and followed by a 4% paraformaldehyde fixative. The cerebrum was then stereotaxically blocked in the coronal plane, removed from cranium, immersed in 0.1 M phosphate buffer, and shipped to Virginia Commonwealth University in a refrigerated container.

2.3 Golgi-Cox Staining

Blocks of cortical tissue containing the auditory cortex were processed using the Rapid GolgiStain Kit (FD NeuroTechnologies, Ellicott City, MD, USA), which was described and specifically recommended for analysis of dendritic spines (Risher et al., 2014) and used in other similar studies (Clemo et al., 2017; Clemo et al., 2016; Clemo and Meredith, 2012). To start, the tissue block was rinsed in double-distilled water and then incubated for 14 days in a dark area at room temperature in a 1:1 mixture of FD solution A/B. The A/B solution was refreshed after the first day. Next, the tissue block was transferred to FD solution C and refrigerated at 4 degrees Celsius in a dark area for 7 days, and the solution was refreshed after first day. After this incubation series, the tissue blocks were sectioned serially (125 μm thickness) on a vibratome, mounted on gelatin-coated glass slides, were reacted for 10 minutes in the D/E solution according to the FD staining procedure. Finally, the selections were dehydrated in a series of alcohols/xylene and coverslipped with Permount. This process is parallel to that used in studies of the primary auditory cortex (Winer, 1984; Mitani et al., 1985; McMullen and Glaser, 1988), of
other auditory cortical regions (Clemo et al., 2016), and in other species (DeFelipe, 2015; Clemo and Meredith, 2012).

2.4 Data Collection

The Golgi-Cox stained tissue sections containing the dorsal zone of auditory cortex (DZ) were first examined using low magnification with light microscopy to find candidate coronal sections with well-stained neurons for subsequent analysis. After selection, the entire tissue sample was traced, followed by identification and labeling of DZ on the lateral lip of the middle suprasylvian sulcus (Stecker et al., 2005; Kok et al., 2013) using Neurolucida (MBF MicroBrightfield, Willston, VT, USA) light-microscope system (Nikon Eclipse 600). Analysis parameters for the area were conservatively set to ensure that only DZ neurons were studied. Next, the grey/white border and laminae were distinguished. Laminar boundaries were determined by local changes in neuron type and density explained in Lee and Winer (2008a, b). Next, the supragranular layers (2 and 3) and infragranular layers (5 and 6) were outlined. Layer 1 was not studied because no identifiable neurons existed, as seen in other neocortical regions. A basis for laminar boundaries was the identification of layer 4, which generally lacks neurons in DZ (Smith and Populin, 2001).

Subsequently, the section would be examined under 40x to identify ideal neurons, which are pyramidal, have identifiable somas, and have intact dendrites with visible dendritic spines. Once selected, the neuronal soma and dendrites were traced. The Neurolucida program allowed for tracking of dendrite type and branch orders. Pyramidal neurons were specifically studied because of their predominance in cortex (~80%) and their characteristic reception of excitatory inputs on their dendritic spines. Their morphology is characterized by a thick, axonal
dendrite oriented towards the pial surface with polarized basilar dendrites that extend horizontally from the base of the neuron. Care was taken to not study inverted pyramidal neurons as they are correlated with inhibitory neurons.

At a higher magnification (1000x, oil), the selected neurons were scanned for apical and basilar dendritic sections. Spine densities are lowest near the neuronal soma, so all dendritic sections studied had to be at least 20 μm from the soma (Elston, 2000). Consequently, data was derived from secondary branch orders or higher. While some dendrites traced up to an 8th branch order, not all reached a high order as a consequence of the plane of section. Ideal sections were chosen, and visible dendritic spines labeled. Sessile and pedunculated spines were identified (according to Stuart et al., 2008) and marked. Filopodic spines were not marked since they are immature, lack a mature synapse, and can’t contribute to excitatory activity. This process was followed for both the control and early deaf groups, and measurements were obtained from similar branch order levels. For each case (n=6), 8-10 supragranular and 8-10 infragranular neurons were identified, and on each neuron, 1-3 apical and 1-3 basilar dendritic spines were counted. In some areas, some neurons were not entirely analyzed because not all neurons carried both axonal and basilar dendrites as a consequence of staining. In these cases, supplemental partial neurons were studied to maintain normalcy of ~120 dendritic sections per case.

Afterwards, using the Contour Mapping function of Neurolucida, a line was applied across the length of dendrite carrying the marked spines to be used later in density analysis. This line was named to identify the particular dendrite and its branch order.

After counting dendritic spines, the dendritic head size was measured using the Neurolucida Quick-measure line tool. Its diameter was measured at the widest dimension when the head was in focus, then the measurement was recorded. Segments of every dendritic section
had calculated average dendritic head size. Finally, data from each neuron was recorded in an Excel spreadsheet according to case, slide, and section number, and the rater was not informed of a given animal’s treatment (early-deaf, hearing) until the spine measures from all animals were complete.

2.5 Data Analysis

Plots of dendritic segments and spines were analyzed using NeuroExplorer (MBF MicroBrightfield, Willston VT, USA) software to determine the length of line and the number of counted spines along that segment. These values were used to calculate spine density (spines/μm) and recorded alongside the laminar location of neuron soma (supragranular or infragranular) and dendritic location (apical or basilar). In addition, the diameter of a selection of marked spine heads was recorded for each dendritic segment. Standard statistical methods were used to determine the average and standard deviation (mean ± SD) of spine densities and head diameter as a function of each variable. All data was examined for normalcy of distribution using a Shapiro-Wilks test; normally distributed sets were compared using a t-test, while non-normal distributed data sets were compared using a Wilcoxon Rank Sum test, and data across multiple groups were compared using ANOVA with post-hoc Tukey's tests (p < 0.05 . significant; JMP Statistical Discovery Software, SAS Institute Inc., Cary NC). For display, tissue sections and representative neurons were reconstructed using camera-lucida (Nikon Eclipse 400 with Y-ITD attachment) and associated dendritic segments were photographed (Nikon Eclipse 600), positioned, and cropped using Photoshop (Adobe Systems).
Results

Golgi-Cox stained tissue sections from dorsal zone of the auditory cortex of hearing (n=3) and deaf (n=3) cats were used to analyze the effects of long-term deafness on dendritic spine density and diameter. Pyramidal neurons (deaf n=70, hearing n=64) were categorized by the laminar location of their cell body as either supragranular (layers 2 and 3) or infragranular (layers 5 and 6). Layers 1 and 4 were not studied because they usually do not contain pyramidal neurons. From this sample, 410 spine-bearing dendritic segments from hearing animals and 416 segments from deaf animals were examined. Figure 2 shows a screenshot of a dendritic segment with its attendant dendritic spines.
Figure 2: Layered screenshots of dendritic segments show dendritic segments with visible spines (1000x magnification, oil).
**Dendritic Spine Density**

A total of 826 of spine-bearing neuronal segments were examined using high magnification (x1,000, oil) and Neurolucida imaging. A summary of the spine densities according to laminar position and dendritic type is supplied in Table 1. When comparing hearing and deaf neurons, the average dendritic spine density (spines per micron) for all DZ neurons from hearing animals (0.99 ± 0.01; mean ± standard deviation (SD)) was significantly greater (T-test, p<0.0001) than the average spine density of those from deaf animals (0.81 ± 0.01) (Figure 3). This trend was maintained when comparing neurons by branch order as well (Table 2). For branch orders 2-6, spine densities were significantly (T-test, p<0.05) higher in hearing animals. This tendency continued for branch order 7 but without statistical significance. For branch orders 8 and 9, spine densities in the deaf animals were actually higher, but the sample size is insufficient for statistical comparison (Figure 4). Since the tissue is prepared in 125 μm sections, it is rare for a neuron to have intact dendritic branches beyond the 7th order. In addition, no dendrites of branch order 1 were sampled because spine density decreases with proximity to the soma (Jacobs et al. 2009).
Table 1

<table>
<thead>
<tr>
<th>Dendritic type</th>
<th>Hearing: density (spines/µm) (mean ± SD)</th>
<th>Deaf: density (spines/µm) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All dendrites</td>
<td>0.99 ± 0.01</td>
<td>0.81 ± 0.01</td>
</tr>
<tr>
<td>(n= 410; 416)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All apical</td>
<td>1.05 ± 0.02</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>All basilar</td>
<td>0.93 ± 0.02</td>
<td>0.77 ± 0.01</td>
</tr>
<tr>
<td>Supragranular</td>
<td>1.00 ± 0.28</td>
<td>0.80 ± 0.23</td>
</tr>
<tr>
<td>Apical</td>
<td>1.08 ± 0.03</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>Basilar</td>
<td>0.93 ± 0.02</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td>Infragranular</td>
<td>0.98 ± 0.02</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td>Apical</td>
<td>1.02 ± 0.03</td>
<td>0.84 ± 0.03</td>
</tr>
<tr>
<td>Basilar</td>
<td>0.94 ± 0.02</td>
<td>0.79 ± 0.02</td>
</tr>
</tbody>
</table>

Table 1: Spine density of pyramidal neurons in DZ by laminar position and dendritic type. n= hearing dendritic segments; deaf dendritic segments
Figure 3: Analysis of spine density (mean ± SD) of neurons from hearing and deaf cats showed that the density in hearing cats was significantly (asterisk, T-test, p<0.0001) higher.
Table 2

<table>
<thead>
<tr>
<th>Branch Order</th>
<th>Hearing: density (spine per µm) (mean ± SD)</th>
<th>Deaf: density (spines per µm) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (n= 68, 66)</td>
<td>0.90 ± 0.03</td>
<td>0.78 ± 0.03</td>
</tr>
<tr>
<td>3 (n= 140, 143)</td>
<td>0.94 ± 0.02</td>
<td>0.79 ± 0.02</td>
</tr>
<tr>
<td>4 (n= 116, 122)</td>
<td>1.06 ± 0.02</td>
<td>0.82 ± 0.02</td>
</tr>
<tr>
<td>5 (n= 42, 59)</td>
<td>1.04 ± 0.04</td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td>6 (n= 22, 14)</td>
<td>1.07 ± 0.05</td>
<td>0.86 ± 0.07</td>
</tr>
<tr>
<td>7 (n= 14, 3)</td>
<td>1.13 ± 0.08</td>
<td>0.86 ± 0.17</td>
</tr>
<tr>
<td>8 (n= 7, 3)</td>
<td>1.01 ± 0.09</td>
<td>1.04 ± 0.14</td>
</tr>
<tr>
<td>9 (n= 2, 3)</td>
<td>0.87 ± 0.14</td>
<td>1.04 ± 0.12</td>
</tr>
</tbody>
</table>

*Table 2: Spine density of pyramidal neurons in DZ by branch order. n=hearing, deaf*
Figure 4: Analysis of spine density (mean ± SD) of neurons from hearing and deaf cats based on branch order (BO) showed that the density in hearing cats for branch orders 2-6 was significantly (asterisk, T-test, p<0.02) higher.
Dendritic Type and Spine Density

For excitatory neurons, apical dendrites course vertically toward the pial surface while basilar dendrites extend horizontally and vertically from the base of the soma. For hearing animals, apical dendrites had a higher average (1.05 ± 0.02) than basilar dendrites (0.93 ± 0.02). This continued in the deaf group where apical dendrites had a higher average (0.85 ± 0.02) than basilar dendrites (0.77 ± 0.01) (Table 1). When comparing hearing and deaf samples, hearing dendrites had a significantly (T-test, p<0.0001) higher density for both apical and basilar types (Figure 5).
Figure 5: Analysis of spine density (mean ± SD) of neurons from hearing and deaf cats based on apical (Ap) and basilar (B) dendritic type showed that the density in hearing cats in both types of dendrites was significantly (asterisk, T-test, p<0.0001) higher than in deaf cats.
Laminar Effects on Spine Density

Since past studies (e.g., Clemo and Meredith 2012; Foxworthy et al. 2013) have demonstrated a relationship between laminar location and spine density, average spine densities were compared based on if the soma was in a supragranular or infragranular layer (Table 1). Neurons from hearing animals had a higher average density in the supragranular layer (1.0 ± 0.28) compared to the infragranular layer (0.98 ± 0.02); deaf neurons did not reflect this pattern (0.80 ± 0.23 and 0.81 ± 0.02, respectively). Even so, laminar spine density from hearing samples was significantly (T-test, p<0.0001) higher than the spine density from deaf samples (Figure 6).

Since the results indicate that dendritic type and laminar position influence the spine density, analysis was taken one step further to compare densities by both parameters (Table 1). For the supragranular layer, neurons from hearing samples had an average apical spine density of 1.08 ± 0.03 and basilar density of 0.93 (± 0.02). The infragranular layer showed the same trend as the apical spine density was 1.02 (±.03) while the basilar density was 0.94 (± 0.02). For neurons from deaf samples, the same pattern continued. In the supragranular layer, apical density was 0.86 (± 0.03) and basilar density was 0.76 (± 0.02). For the infragranular layer, apical density measured 0.84 (± 0.03) while basilar density was 0.79 (± 0.02). In comparing hearing and deaf in each category, the densities from hearing samples were significantly (T-test, p<0.0001) higher than those from deaf samples (Figure 7).

In summary, dendritic spine density was significantly reduced for DZ neurons of deaf animals across all measures of laminar and dendritic location.
Figure 6: Analysis of spine density (mean ± SD) of neurons from hearing and deaf cats based on neuronal laminar location showed that the density in hearing cats in the supragranular (SG) and infragranular (IG) layers was significantly (asterisk, T-test, p<0.0001) higher.
Figure 7: Analysis of spine density (mean ± SD) of neurons from hearing and deaf cats based on apical (Ap) and basilar (B) dendritic type and neuronal laminar location (SG, IG) showed that the density in hearing cats in all combinations (apical supragranular, basilar supragranular, apical infragranular, basilar infragranular) was significantly (asterisk, T-test, p<0.0001) higher.
Dendritic Spine Head Diameter

Since previous studies have demonstrated a correlation between plasticity and dendritic spine size (e.g., Trachtenberg et al. 2002; Kasai et al. 2003), the diameters of the widest portion of the spine head from 7872 hearing and 6354 deaf samples were collected and compared (Table 3). Significant differences in the average diameter were present but not constant across all parameters. As seen in Figure 8, comparing hearing and deaf samples showed a significant (T-test, p<0.0054) difference with the deaf samples having a larger average diameter. When branch order was taken into account, spines from deaf samples were mostly larger than the hearing counterparts (Table 4). For branch order 2, the spine size of 0.50 (± 0.14) in deaf animals was significantly (T-test, p<0.0028) greater than the size of 0.48 (± 0.15) in hearing animals. Similarly, for branch order 5, the size of deaf spines (0.51 ± 0.15) was significantly (T-test, p<0.0014) greater than the size of hearing spines (0.50 ± 0.14) (Figure 9).
Table 3

<table>
<thead>
<tr>
<th>Dendritic type</th>
<th>Hearing Spine diameter (µm) (mean ± SD)</th>
<th>Deaf Spine diameter (µm) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=7872; 6354)</td>
<td>0.497 ± 0.14</td>
<td>0.504 ± 0.14</td>
</tr>
<tr>
<td>Apical</td>
<td>0.492 ± 0.15</td>
<td>0.499 ± 0.14</td>
</tr>
<tr>
<td>Basilar</td>
<td>0.501 ± 0.14</td>
<td>0.509 ± 0.14</td>
</tr>
<tr>
<td>Supragranular</td>
<td>0.4999 ± 0.14</td>
<td>0.502 ± 0.14</td>
</tr>
<tr>
<td>Infragranular</td>
<td>0.493 ± 0.15</td>
<td>0.505 ± 0.14</td>
</tr>
</tbody>
</table>

Table 3: Spine head diameter neurons in DZ by laminar position and dendritic type. n= hearing spines; deaf spines
Figure 8: Analysis of spine diameter (mean ± SD) of neurons from hearing and deaf cats showed that the density in deaf cats was significantly (asterisk, T-test, $p<0.0054$) higher.
Table 4

<table>
<thead>
<tr>
<th>Branch Order</th>
<th>Hearing: diameter (µm) (mean ± SD)</th>
<th>Deaf: diameter (µm) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (n= 1152, 1029)</td>
<td>0.482 ± 0.15</td>
<td>0.501 ± 0.14</td>
</tr>
<tr>
<td>3 (n= 2545, 2125)</td>
<td>0.503 ± 0.00</td>
<td>0.502 ± 0.00</td>
</tr>
<tr>
<td>4 (n= 2387, 1873)</td>
<td>0.498 ± 0.00</td>
<td>0.503 ± 0.00</td>
</tr>
<tr>
<td>5 (n= 926, 919)</td>
<td>0.491 ± 0.14</td>
<td>0.512 ± 0.15</td>
</tr>
<tr>
<td>6 (n= 415, 246)</td>
<td>0.501 ± 0.14</td>
<td>0.507 ± 0.13</td>
</tr>
<tr>
<td>7 (n= 257, 48)</td>
<td>0.490 ± 0.15</td>
<td>0.485 ± 0.13</td>
</tr>
<tr>
<td>8 (n= 130, 60)</td>
<td>0.503 ± 0.14</td>
<td>0.495 ± 0.16</td>
</tr>
<tr>
<td>9 (n= 26, 41)</td>
<td>0.565 ± 0.14</td>
<td>0.529 ± 0.12</td>
</tr>
</tbody>
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Table 4: Spine head diameter neurons in DZ by branch order. n= hearing, deaf
Figure 9: Analysis of spine diameter (mean ± SD) of neurons from hearing and deaf cats based on branch order (BO) showed that the density in deaf cats was significantly (asterisk, T-test, p<0.0028) higher for only second and fifth branch order.
**Dendritic Type and Spine Head Diameter**

Spine sizes were also compared based on apical and basilar dendritic type (Table 3). Although the spine size of deaf samples was greater than those from hearing cases in both apical and basilar types, only the latter had a significant difference (T-test, \( p<0.0245 \)) (Figure 10). For the deaf samples, the apical average was 0.49 (± 0.15) while the basilar average size was 0.50 (± 0.14). In comparison, for the hearing samples, the apical and basilar average size was 0.50 (± 0.14).
Figure 10: Analysis of spine diameter (mean ± SD) of neurons from hearing and deaf cats based on apical (Ap) and basilar (B) dendritic type showed that the density in deaf cats was significantly (asterisk, T-test, $p<0.0245$) higher for only basilar types.


*Laminar Effects on Spine Head Diameter*

Following the trend of the above analyses, spine diameter was compared based on the supragranular or infragranular laminar position of the parent soma (Table 3). Although the deaf samples maintained a higher average spine size than the hearing samples in both positions, there was only a statistical difference in the infragranular layer (T-test, p<0.0007) (Figure 11). In the deaf samples, the supragranular average was 0.50 (± 0.14) while the infragranular average size was 0.51 (± 0.14). In comparison, for the hearing samples, the supragranular average was 0.50 (± 0.14) while the infragranular average was 0.49 (± 0.14).

In summary, small but significant increases in the average spine head diameter were present in DZ neurons of deaf animals, but these effects were not constant across all parameters.
Figure 11: Analysis of spine diameter (mean ± SD) of neurons from hearing and deaf cats based on apical (Ap) and basilar (B) dendritic type showed that the density in deaf cats was significantly (asterisk, T-test, $p<0.0245$) higher for only basilar types.
Discussion

It has been well established that sensory deprivation is associated with cross-modal changes in the brain. After auditory deprivation, remaining sensory modalities recruit auditory areas, causing compensatory changes. In response to deafness, regions of the auditory cortex become responsive to somatosensory (Levänen et al., 1998; Levänen and Hamdorf, 2001; Allman et al., 2009; Bhattacharjee et al., 2010; Meredith and Lomber, 2011; Karns et al., 2012) or visual stimulation (Neville et al., 1983; Finney et al., 2001, 2003; Lee et al., 2001; Lambertz et al., 2005; Pekkola et al., 2005; Lomber et al., 2010; Meredith et al., 2011; Karns et al., 2012).

Although many examples of plasticity due to sensory deprivation have been examined, the mechanisms behind these changes and the neural basis of behavioral compensation remain largely unknown. In 1995, Rauschecker proposed three plausible means. After a major sensory system is deactivated, there may be increased projections from existing sources, enhanced ingrowth of new projections, or ‘unmasking’ of existing inputs that were silent before. These mechanisms have been reiterated in many studies. Perhaps the best studied model is the reorganization of sensory maps due to changes in local connectivity. This includes local sprouting and the ‘unmasking’ of silent inputs (Bavelier and Neville, 2002). Anatomical studies in cats and non-human adult primates showed existing, direct connections between the auditory and visual cortices (Hall and Lomber, 2008; Falchier et al., 2002; Rockland and Ojima, 2003). These existing connections provide the possibility that sensory deprivation leads to more pronounced changes in the connectivity between those cortices (Merabet and Pascual-Leone, 2010). There is also evidence supporting that changes in cortico-cortical connectivity are responsible. Wittenberg, et. al. (2004) demonstrated connectivity between the visual and somatosensory cortex areas through TMS and PET imaging. Other studies involving rapid and
complete visual deprivation using a blindfold on seeing individuals showed rapid and reversible recruitment of the occipital lobe (Merabet et al., 2008). These last findings are congruent with the ‘unmasking’ of present but silent inputs. This mechanism could be responsible for early, rapid changes that can lead to long-term and more permanent changes like dendritic branching, sprouting, and rewiring of connections (Pascual-Leone et al., 2005) and is certainly more plausible than mechanisms involving growth and guidance of new inputs from distant brain areas.

Of particular interest for this study was the dorsal zone (DZ), an associative area of the primary auditory cortex (A1). DZ is known to participate in cross-modal plasticity following deafness. In a study by Lomber et al. (2010), behavioral testing showed that deaf cats had enhanced movement detection through lowering of the motion discrimination threshold. Through targeted individual cooling loop deactivation, DZ was found to be the area responsible for this change in visual perception. A study by Barone and Kral (2013) verified anatomical reorganization of connectivity in DZ through the injection of retrograde dyes. They found that the DZ area of deaf cats received a small number of ‘abnormal’ non-auditory inputs from the ventral posterior ectosylvian gyrus, the multimodal SIV/orbito-frontal regions, certain visual areas, and the anterior medial later suprasylvian area. These new projections from various areas provide routes for non-auditory information into DZ, which could strengthen the visual reorganization of the area (Barone and Kral, 2013). However, these new connections are only a small percentage of the total inputs to DZ and are weak connections. Therefore, there are most likely other mechanisms at play as well. It is possible that the functional reorganization is supported by the normal network of the auditory system itself since several sources of visual inputs have indirect contact with the auditory cortex. It is most likely that the cross-modal
compensation is due to the ‘unmasking’ or increased efficiency of multimodal connections linking the auditory cortex to cortical or thalamic structures involved in visual and tactile processing (Kujala et. al., 2000; Wong and Bhattacharjee, 2011; Klinge et. al., 2010; Merabet and Pascual-Leone, 2010).

Since pyramidal dendritic spines are the receivers of excitatory input, spine density is another experimental method to analyze input into a cortical area. The decrease in spine density fits with Rauschecker’s various methods of cross-modal plasticity. Because data from connectional studies (Barone & Kral, 2013; Kok et al., 2014) show a non-significant reduction in projections from A1 (and other auditory cortices) to DZ, we expect to see a decrease in spine density (as demonstrated by the present study), although some projections between A1 and DZ may be maintained in early deafened animals (Meredith and Allman, 2012; Barone et. al., 2013; Chabot et. al., 2013; Kok et. al. 2014; Meredith et. al., 2013). Based on this literature, it seems safe to argue that reduced auditory cortical inputs to DZ could account for a slight but consistent spine loss in DZ (Kok et. al. 2014).

From behavioral studies, we know that DZ is being recruited for visual use in the deaf (Lomber et al., 2010; Land et al., 2016). As a consequence, the remaining spines on DZ neurons could receive input from novel projections from visual and somatosensory areas, although these novel inputs are only a small percentage of total inputs (Barone and Kral, 2013). Given the known presence of cross-modal plasticity in DZ from behavioral and retrograde dye studies, we know at least one of Rauschecker’s methods is at play. Since it is unlikely that the changes observed are solely from novel input (Barone and Kral, 2013), it is apparent that silent inputs were unmasked from the non-auditory pathways to DZ already present prior to deafening.
With regards to spine diameter, larger spine heads are associated with stable, mature neural circuits (Trachtenberg et. al., 2002; Kasai et. al., 2003). Moreover, activation that induces long-term potentiation is also correlated with spine enlargement. Therefore, spine head diameter is a well-studied and robust indicator of excitatory synapse maturity and neuronal circuit plasticity. These results found that spine heads from dendrites of deaf subjects were sometimes statistically larger, but overall, the diameters were quite similar. The general similarity in dendritic spine diameter between the deaf and hearing cases suggest that the spines receiving inputs from cross-modal signaling in the early deaf have the same stability and efficacy as those involved in normal developmental conditions. Since deaf dendrites did carry spines that had slightly larger average diameters than those from hearing dendrites, it is possible that cross-modally reorganized areas lose some potential for additional plasticity, which could be the consequence of a reduced variety of input into the region. Since the average spine diameter of deaf spines is only very slightly increased, it seems most likely that these changes would not have a profound physiological effect in deaf animals.

In moving forward with studies on cross-modal plasticity as a result of sensory deprivation, it is important to keep in mind the clinical implications of these findings. As we continue to understand the mechanisms behind plasticity, we gain a greater understanding of critical periods, when to medically intervene, and rehabilitation. It is relatively unknown how a repurposed sensory area can revert to its original function following medical intervention, such as cochlear implants. In a comparable study studying loss of sight, patients with treatable early-onset vision found visual tasks, such as identification and recognition of objects, particularly difficult after their vision was restored (Fine et. al., 2003). With further studies, these results propose that different visual areas that process different visual attributes could vary in their
susceptibility to visual deprivation and in their recovery rates (Fine et. al., 2002; Fine et. al.,
2003; Ostrovsky et. al. 2006; Saenz et. al., 2008). Studies like these, although centered
around loss of vision instead of loss of hearing, allow us to examine the brain’s response to
loss of sense and its response to medical intervention. Observations from these studies could
have a lasting impact on the approach and strategy of visual rehabilitation. Although cross-
modal plasticity is well documented, the mechanism behind the neural changes is still
relatively unknown. By approaching the analysis of these changes in all directions, like with
spine density and spine head diameter evaluation, we are able better understand the mystery
behind how the brain compensates for loss of sensory function through cross-modal plasticity.
References


Vita

Rachel J. Bauer, maiden name Gilbert, was born in Hollywood, Florida in 1992. She did her undergraduate study at the University of Virginia, where she graduated with a B.A. in chemistry and a B.S. in Spanish linguistics and philology in 2015. During undergraduate research, she was published as second author in Analytica Chimica Acta (Thompson et. al. (2016) Hematocrit analysis through the use of an inexpensive centrifugal polyester-toner device with finger-to-chip blood loading capability. Analytica Chimica Acta 924:1-6)). Rachel enrolled in the VCU post-baccalaureate program in 2017, and after completion, transitioned into the Master’s of Anatomy and Neurobiology.