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# **Synaptic Plasticity in A Visual Cortical Region Induced by Early-Deafness**

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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B.S., Villanova University, 2016

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## Abstract

### Synaptic Plasticity in A Visual Cortical Region Induced by Early-Deafness

By John Michael Kay, B.S.

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When organisms learn and adapt to their environment or lose a sensory modality, neurons in the brain undergo a cellular process called ‘plasticity.’ This thesis explores the loss of a non-visual system (early deafness) and how it can affect visual plasticity. To examine this question, Golgi-stained cortical neurons were studied from the visual region PLLS from early-deaf cats and their hearing controls. Dendritic spine density and dendritic spine diameter are well-known indicators of synaptic plasticity and these neuronal features were measured using light microscopic techniques and NeuroLucida. Within the visual PLLS, the mean spine density for the deaf cats was  $1.171 \pm 0.295$  spines/micron, while for hearing cats it was  $0.984 \pm 0.227$  spines/micron, which was a statistically significant increase ( $p < 0.0001$ ). The mean spine diameter for the deaf cats was  $0.478 \pm 0.119$  microns, while for hearing cats it was  $0.527 \pm 0.211$  microns, which is a statistically significant decrease ( $p < 0.0001$ ). These changes in dendritic spine properties indicate that the neurons in the PLLS underwent synaptic plasticity. These findings are significant because they show that visual regions of cortex can be affected by non-visual conditions or treatments such as early deafness.

## Introduction

Plasticity is a cellular process and concept that the brain is malleable when it comes to adapting to its environment. This malleability allows an organism to learn from past mistakes (ex. eating a bitter fruit) and make new strategies (ex. catching prey). However, plasticity comes in many different types and forms and can be affected in different ways. One of the most interesting types is “cross-modal” plasticity. Cross-Modal plasticity can occur when an organism loses a major sensory modality, such as hearing, and the brain compensates for this lack of sensory input by recruiting neuronal projections and synapses from other sensory modalities (Lomber, Meredith, and Kral 2010). It has been well documented that cross-modal plasticity can lead to an organism performing certain tasks in the intact sensory modalities better than healthy subjects (Renier, De Volder, and Rauschecker 2014; Frasnelli et al. 2011; Merabet and Pascual-Leone 2010; Teichert and Bolz 2017; Gougoux et al. 2005) due to its reorganization of neuronal projections. However, in order to measure cross-modal plasticity, it is necessary to examine the functional unit of the nervous system, neurons.

Neurons transmit electrical and chemical signals from one area of the body to the other. This electrical impulse is the method in which our body communicates to our brain about our external and internal environment. In order to do this effectively, the neuron developed three distinct components in order to carry out its function. The first component is the "cell body" or the "soma." It is where the nucleus of the neuron is housed as well as other organelles to help keep the neuron alive. The soma produces neurotransmitters, which are used as chemical signals for the neuron to use as another form of communication, as well. The second segment is the "axon." The axon is the component of the neuron that sends an electrical impulse away from the neuron towards other neurons or structures and does not branch until near its termination. The third compartment is the dendrite. The dendrite's primary role is to receive signals and transmit

them down to the cell body. Unlike the axon, the dendrite can have a multitude of branches. The abundance of branches allows the dendrite to synapse with axons from different neurons. On these dendrites, are even smaller units that protrude from dendrites. These smaller units are named "dendritic spines."

A dendritic spine is a protrusion of the dendritic membrane that can create synapses with axons that carry excitatory neurotransmitter. A dendritic spine also contains a neck which attaches the head of the spine to the dendrite itself. There are four types of dendritic spines: "Stubby," "Thin," "Mushroom (or Pedunculated)," and "Filopodium" (Peters and Kaiserman-Abramof, 1970). A "thin" dendritic spine is characterized by a thin neck with a large head. A "mushroom" dendritic spine is characterized by a large head and a thicker neck. A "stubby" dendritic spine is characterized by a head and no visible neck to it (Jones and Powell 1969; Peters and Kaiserman-Abramof 1970; Yuste, 2010, pp. 24). These first three dendritic spine conformations are all considered mature. Because these three spine shapes have not been demonstrated to serve different or distinct functions, many recent studies do not distinguish between them (e.g., Clemo and Meredith, 2012; Clemo et al., 2016; 2017). On the other hand, "filipodium" are immature dendritic spines which have no visible head and instead look more like cilia. Filipodia are in the process of developing or retracting and generally do not support synaptic contacts with axons. A final characteristic of dendritic spines is that they contain no rough endoplasmic reticulum (RER), mitochondria, or microtubules (Yuste, 2010 pp 30-31). Instead, they contain polyribosomes for protein synthesis and actin for support (Yuste, 2010 pp 29, 31).

The primary function of a dendritic spine is to make synapses with excitatory axon terminals and, thereby, transmit excitatory signals from presynaptic to postsynaptic neurons. A

study done by Matsuzaki et al. (2004) shows that dendritic spines increase in size and number following lengthy repeated stimulation, known as long term potentiation. However, if a neuron is not stimulated, the dendritic spines on the neuron will regress in both size and number.

Therefore, dendritic spines are capable of dynamically responding to a variety of environmental and disease states. For example, squirrels lose about 40% of the dendritic spine volume during hibernation but recover that loss once they wake up (Popov and Bocharova 1992; Popov, Bocharova, and Bragin 1992). There are also other mechanisms and processes that can increase or decrease the amount of dendritic spines a neuron can produce. One such process is circadian rhythm and light, which can regulate not only the number of spines, but also regulate the amount of synapses a dendritic spine can produce (Jasinska et al. 2019). Another factor that can regulate spine density is dopamine (Alberquilla et al. 2020). Growth hormone (Nylander et al. 2020), environmental richness (Kolb, Cioe, and Comeau 2008), and exercise (Stranahan, Khalil, and Gould 2007) can also increase spine number. Just as some internal and external factors can increase spine numbers, other factors can decrease spine numbers. These factors include genetic issues such as Alzheimer's (Gutierrez et al. 2019; Kim et al. 2020), trisomy-21 (Purpura 1974), fragile X syndrome (Comery et al. 1997), and schizophrenia (Garey et al. 1998; Glantz and Lewis 2000). However it has been found that even stress (Bose et al. 2010), and drug abuse (Berman et al. 1996; Mei et al. 2009), can lead to a decrease in spine numbers as well. With these factors, it becomes obvious that not only can neurological diseases change dendritic spine number, but also everchanging events such as stress or metabolic cycles can affect dendritic spines as well. These factors highlight the plasticity of dendritic spines.

Since many outside factors can influence the number of dendritic spines, it is important to understand what normal development of a dendritic spine looks like. During development,

exuberant numbers of dendritic spines are expressed (Ramon y Cajal 1904). However, after this initial surge of dendritic spines a ‘pruning process’ (Ramon y Cajal 1904) begins in order to remove ineffective or inactive synapses. This period of spine pruning is a time in which the nervous system “tunes” itself to the features of the environment that activate it, called the “critical period of development”. This critical period is a fixed window and once the critical period ends, it does not return for the rest of the organism’s life. For example, the auditory critical period for cats is from day 50 postnatal (Kral et al. 2005) to day 80-100 postnatal (Kral 2013). For humans, studies done on the development of hearing and cochlear implantation in young children suggest that the first seven years of life is the auditory critical period (Niparko et al. 2010; Sharma, Dorman, and Spahr 2002; Sharma et al. 2002; Sharma, Dorman, and Spahr 2002). Thus the spines that are retained and persist into maturity are those which are active. It should be noted however, that during an organism’s sensory critical period, dendritic spine development can be drastically altered by abnormal or deprived sensory conditions.

Special sensory conditions, such as monocular deprivation, are known to have drastic effects on brain and neural development, as shown by the work of Nobel Laureates Hubel and Wiesel (Hubel, Wiesel, and LeVay 1977). This work showed that loss of sensory activity from one eye dramatically rearranged the structure and function of areas of visual cortex and its neuronal connections. Specifically, the input connections to cortical layer four from the deprived eye were substantially reduced while those from the active eye expanded (Hubel, Wiesel and LeVay, 1977). Similarly, early hearing loss (before the auditory critical period) deprives auditory cortex of activation, and results in cross-modal plasticity of the deprived areas of auditory cortex (Lomber, Meredith, and Kral 2010; Meredith and Lomber 2011; Meredith et al. 2011). Furthermore, the cross-modal effects of early deafness affects the expression of dendritic spine

properties in auditory cortices such as the A1 and FAES regions of the auditory cortex (Clemo, Lomber, and Meredith 2016; 2017). Clemo (2016; 2017) found that early deafness caused an increase in spine number and spine head size in the supragranular (SG) layer of these regions. These studies show that hearing loss has specific effects on neural connections and processing in areas of the brain that would have processed auditory information. However, it is not known whether hearing loss can affect neural connections and processing in areas of the brain that process non-auditory information, such as visual areas.

Within the visual cortex in a cat, there is a region called the lateral suprasylvian visual area (LS) (Figure 1A). The LS demonstrates a variety of visual functions that help the cat interact with its environment, mainly in the form of visual motion processing (Kiefer et al. 1989; Spear and Baumann 1975; Krüger et al. 1993). Within the LS there are six subregions: the anteromedial suprasylvian area (AMLS), the anterolateral lateral suprasylvian area (ALLS), posterior medial-lateral suprasylvian (PMLS), posterior lateral lateral suprasylvian area, dorsal lateral suprasylvian area (DLS), and the ventral lateral suprasylvian area (VLS) (Palmer, Rosenquist, and Tusa 1978). With these regions in mind, the part of the LS that will be examined in this project is the posterolateral lateral suprasylvian area (PLLS).

The PLLS is located within the caudal, lateral two-thirds wall of the suprasylvian sulcus (Palmer, Rosenquist, and Tusa 1978)(as seen in Figure 1B and 1C) and receives extensive projections from visual cortices 17, 18, 19, 20, and 21 (Symonds and Rosenquist 1984; Norita et al. 1996; Sherk 1986). Neurons in the PLLS are involved in motion processing and exhibit directional selectivity (Rauschecker, von Grunau, and Poulin 1987; Spear and Baumann 1975). More specifically, PLLS neurons are more sensitive to visual stimulus movement in directions away from the midline and towards the bottom right quadrant of the visual field (Rauschecker,

von Grunau, and Poulin 1987). It was also found to play a role in optic flow processing and respond better to radial motion (Li et al. 2000) which are components of visual motion. Ultimately, lesions that damage the PLLS produce deficits in visual orienting and localization behaviors (Hardy and Stein 1988). The summary function of the PLLS is visual motion processing (Rauschecker, von Grunau, and Poulin 1987; Robitaille et al. 2008).

It has been found that the PLLS not only receives projections from visual cortices, but from auditory cortices as well (Clemo et al. 2008). These auditory cortices include the DZ, A1, AII, and FAES (Clemo et al. 2008). The DZ, A1, and AII all provide tonal information (Reale and Imig 1980; Schreiner and Cynader 1984; Stecker et al. 2005), with A1 also involved with the function of auditory localization (Middlebrooks, Dykes, and Merzenich 1980). All three of these regions were found to project heavily into the PLLS/DZ border and projected less so to the bank of the sulcus as well as its posterior levels (Clemo et al. 2008). The FAES region has two functions; receiving auditory stimuli with complex frequency properties (Clarey and Irvine 1986), and receiving auditory stimuli with spatial properties (Las, Shapira, and Nelken 2008; Middlebrooks et al. 1994). Unlike the DZ, A1, and AII regions, the FAES projections were found to project consistently throughout the PLLS, but project more strongly in the SG layer (layers 2 and 3) (Clemo et al. 2008). With these auditory projections to the PLLS, there is functional evidence that the PLLS, a visual region, is affected by these auditory projections.

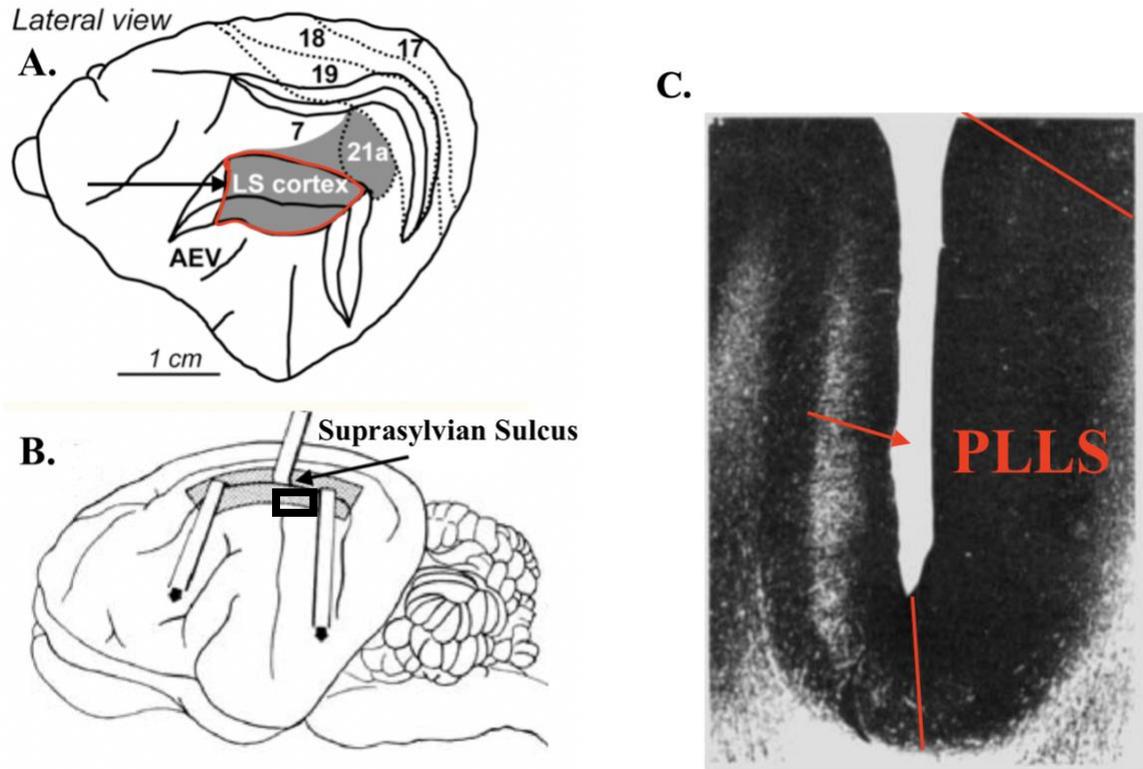


Figure 1: A. Lateral view of the location of the lateral suprasylvian (LS) area (grey patch within the red border and denoted by the arrow) in relation to other visual areas of the cat cortex. Modified from Huxlin, Williams, and Price (2008). B. Within the gray area (that corresponds with the gray area in A) is the suprasylvian sulcus (denoted by the arrow) being opened, with the posterolateral lateral suprasylvian sulcus (PLLS) region exposed (denoted with the black box). Modified from Palmer et al. (1978). C. This figure is a modified figure that is found in Palmer et al. (1978) and shows an enlarged view of the Posterolateral Lateral Suprasylvian area (PLLS) along the wall of the suprasylvian sulcus (denoted by the red arrow).

Three functional types of neurons are found within the PLLS. Unimodal visual neurons are the most abundant and only respond to visual inputs. The second type of neurons in the PLLS are bimodal neurons. Bimodal neurons may be activated from two separate sensory modalities (ex. auditory and visual) (Yaka et al. 2002). The third type of neurons found in the PLLS is the subthreshold neuron. In a study by Allman et al. (2007), subthreshold neurons were found to react to visual input and not to auditory input alone, however the authors found that the visual responses of these neurons could be modified by auditory inputs. Auditory bimodal and subthreshold responses are consistent with the auditory projections that terminated in the PLLS. However, these two neurons were not distributed equally throughout the region but instead were found to be separated from each other as described below.

It has been shown that a majority of bimodal neurons congregated in the upper third of the PLLS, while subthreshold neurons congregated more in the lower two-thirds (Allman and Meredith 2007). One explanation for this separation (Clemo et al. 2008) is that the upper third of the PLLS receives inputs from the primary auditory cortices AI, AII and DZ (Clemo et al., 2008) which could give rise to the presence of bimodal neurons in this location (He et al. 1997; Clemo et al. 2008; Stecker et al. 2005). Because the DZ, A1, and AII all terminate more strongly at the upper third of the PLLS, there is a possibility that these strong projections correlate with the congregation of bimodal neurons in the upper region (He et al. 1997; Clemo et al. 2008; Stecker et al. 2005). However, the lower region of the PLLS, receives much smaller auditory projections (Clemo et al. 2008) and may still have some type of influence (Allman and Meredith 2007). These weak but still influential, auditory projections may correspond to subthreshold neurons congregating in the lower two-thirds of the PLLS. In addition, these auditory regions project

mainly into the SG layer of the PLLS which can possibly lead changes in the PLLS if deafness occurs.

The results of the aforementioned research suggest that the PLLS is a region that receives both visual and auditory inputs. However, it is unknown what occurs to this region when it loses auditory stimuli following hearing loss. This question is addressed in the following study.

## Materials and Methods

The present study used a collection of brain tissue obtained after the completion of other experiments at the University of Western Ontario and histologically processed at Virginia Commonwealth University. All procedures for the handling of the animals followed the guidelines of 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) were approved 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.

The cortical tissue used in this experiment was derived from seven unspayed female adult cats that were at least eight months of age whose vital statistics are summarized in Table 1. The use of only female cats was an unplanned consequence of the experimental schedule of collaborators at Western Ontario University, who provided the brain tissue from hearing and from early-deaf cats. Cats that were included were required to reach at least eight months of age. This age bar was chosen because cats reach maturity at six months postnatal (Kral et al., 2005), and this inclusion criteria avoids the well-known effects of developmental plasticity. Tissue from a total of seven cats was examined in this study: three hearing control cats and four early-deaf. The additional early-deaf cat was necessary because in one case (#321) stained neurons that met study criteria were not available to be examined (possibly as an artifact of the staining procedure), and selected neurons from another early-deaf case (#204) were used to complete the sample. Values from case #204 neurons were sufficiently similar to those derived from the other layers in case #321 that the data was fused. Values for these and other cases are detailed in Table

2, where the contribution from case #204 is listed separately from that of #321 but are thereafter combined.

Case Number	Deaf vs. Hearing	Age	Sex	Weight (kg)
147	Deaf	17 months	F	3.1
150	Hearing	24 months	F	4.3
163	Hearing	12 months	F	4.1
164	Deaf	9 months	F	2.9
204	Deaf	16.4 months	F	2.3
211	Hearing	38 months	F	3.1
321	Deaf	27.7 months	F	2.6
Deaf Avg		17.525 months	F	2.725
Hearing Avg		24.66 months	F	3.833

Table 1: Summary of the vital statistics and treatment for each cat whose cortical tissue was examined in the present study.

At the University of Western Ontario, hearing loss was induced in 15-30 day old cats using a single dose of sodium edecrin coadministered with kanamycin after the methods of Xu et al., (1993). This timeline was selected to produce an auditory lesion before the auditory critical developmental period for cats, which begins at 50 days after birth (Kral et al. 2005). The treated cats were confirmed to be deaf when they showed a lack of auditory brainstem responses (ABR) as shown in other studies of early-deaf cats (Clemo, Lomber, and Meredith 2016; Kok, Chabot, and Lomber 2014; Wong et al. 2015). In contrast, the three hearing control cats demonstrated normal ABR responses. After reaching adulthood, the cats were euthanized with 100 mg/kg of sodium pentobarbital intravenously. Then they were perfused with 4% paraformaldehyde. The brain was then stereotaxically blocked in the coronal plane, was submerged with 0.1 M phosphate buffer, and then shipped to VCU in a refrigerated state.

Histological processing occurred at VCU where the selected cortical tissue underwent incubation and processing to produce a Golgi stain using a FD Rapid GolgiStain kit (FD Neurotechnologies, Columbia, MD). First, the block of tissue was washed with double-distilled water and incubated in a dark room for 14 days at room temperature in a 1:1 mixture of FD solutions A/B. Then, the block of tissue was immersed in FD solution C for seven days in the dark at 4 degrees Celsius. After completion of the incubation series, the block of tissue was sectioned serially at 125 microns thick on a vibratome. The tissue samples were then mounted from FD solution C onto gelatin-coated glass slides and allowed to air-dry overnight in a dark room. Finally, the sections were reacted using FD staining solution D for 10 minutes to produce the visible reaction product. Last, these sections were dehydrated in a series of alcohols and xylene and were coverslipped with Permount.

Light microscopy was used to view and examine the Golgi-stained neurons. This involved a Nikon Eclipse E600 microscope connected to a PC-computer equipped with the NeuroLucida program (MBF Bioscience, Williston, VT) to control a 3D digital stage. The rater/microscopist was blinded to the hearing/early-deaf status of the animal from which the tissue was derived until the data collection phase was complete. First, tissue sections containing the PLLS were viewed under low magnification to assess the presence and distribution of Golgi-labeled neurons. If a section was found suitable for study, the entire tissue section was mapped under low-magnification (10x objective) as were the borders, the upper/lower subregions and layers of the PLLS. Next, Golgi-stained neurons were sought in each lamina that met the following criteria: neurons with pyramidal morphology evidenced by a singular apical dendrite (oriented toward the pia), a sufficiency of dendritic spines and little to no artifact (Figure 2A). Once the distribution of candidate pyramidal neurons for study was identified, individual neurons were selected for tracing at higher magnification (40x objective). A scaled, digital tracing of a neuron, its cell body and its dendritic branches was made using NeuroLucida, similar to that shown in Figure 2B. For these tracings, apical dendrites were defined as a distinct, thick trunk oriented towards the pial layer, while basilar dendrites were often smaller, multiple dendritic trunks that extended on the opposite side of the apical dendrite. As a dendritic tracing progressed, the NeuroLucida program automatically kept track of dendritic branch order levels. Next, individual dendritic segments were selected for spine measurements based on exhibiting clear dendritic spines, having more than 10+ dendritic spines on a segment that was at least 20 microns long. Next, along the selected segment, dendritic spines were visualized and marked based on if they had round heads and had a stem attaching to the parent dendrite. Last, spine head diameters were measured by using the “quick measure tool” in the NeuroLucida program. A

representative example of a reconstructed pyramidal neuron from the PLLS, with its dendritic branches and selected dendritic spine markers, is illustrated in Figure 2B.

For each case that was analyzed, dendritic segments were mapped and dendritic spines were measured. This was done on both apical or basilar dendrites to check for similar numbers of neurons in each layer of both the upper third PLLS sub-region and the lower two-thirds subregion. This ensured, a balanced set of measured features was obtained that totaled approximately 100 segments per case (Table 2). Values for spine counts, dendritic segment length (from which spine density was calculated; spines/micron), and spine diameter were imported from Neurolucida into an Excel spreadsheet, where these measures were tabulated with the laminar position (supragranular versus infragranular), dendritic segment type (apical versus basilar), dendritic segment branch order, and PLLS subregion (upper versus lower). In this way, measures of spine density and spine diameter could be compared across different conditions and hearing status. It was not until the data was documented and tabulated in Excel the rater/microscopist was informed of the hearing status of the different cases (hearing controls versus early-deaf).

Analysis consisted of using the Excel program to calculate means ( $\pm$  standard deviation) of selected features. Values obtained for different groups of data were statistically compared using a t-test and statistical significance was defined as  $p < 0.05$ . The Excel program was also used to create graphs for the visual display of the results.

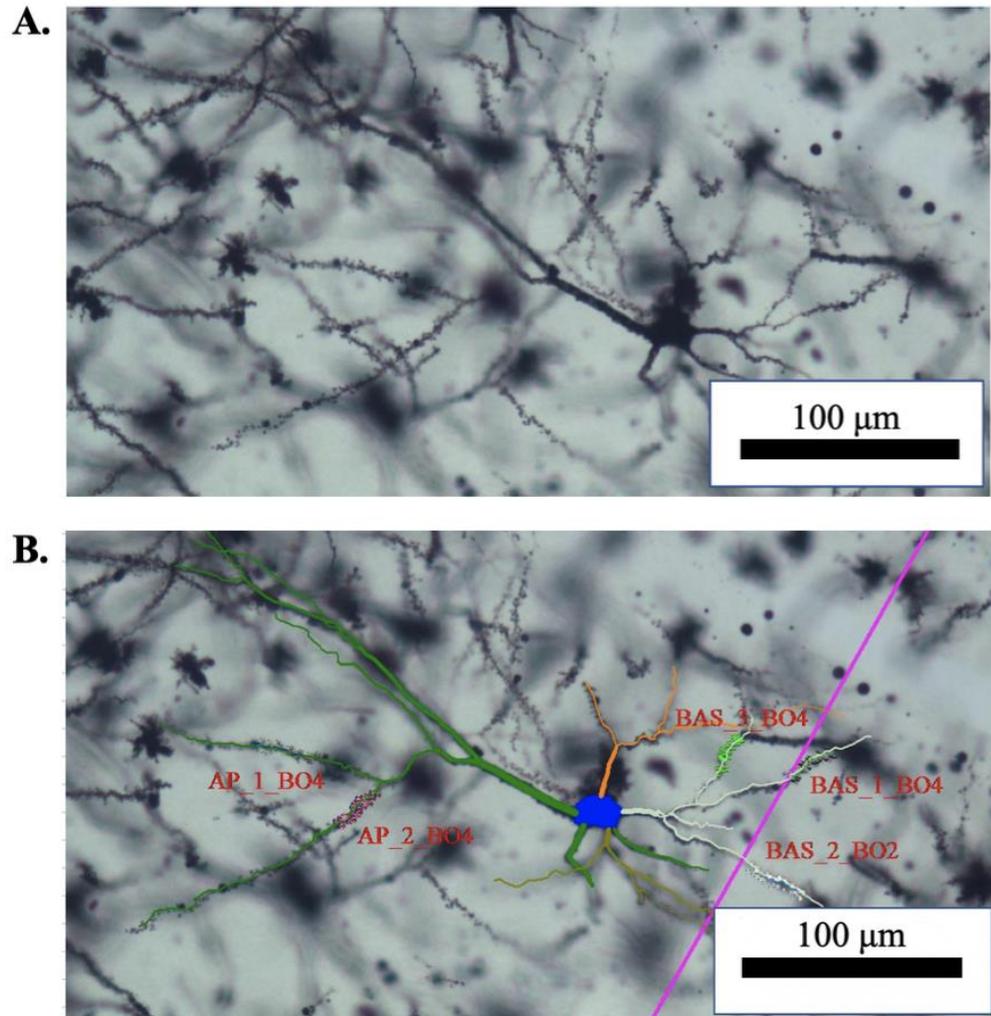


Figure (2): A. This graphic shows a Golgi-stained pyramidal neuron. B: This graphic shows the neuron's superimposed tracing (using Neurolucida). The apical dendrite is the left-most branch (green) from the cell body (blue) and is oriented towards the pia (top left side of the figure). Basilar dendrites (orange, white, and olive green) are depicted toward the right side of the cell body. Selected dendritic segments (indicated by the text labels) were examined under higher power (100x objective, oil) to mark and measure the dendritic spines. Markers for dendritic spines are apparent as the small colored dots lined up on each side of a dendritic segment. The diagonal pink line represents a laminar boundary of the PLLS.

## Results

This study evaluated the role of dendritic spine plasticity (as measured in terms of dendritic spine density and spine head diameter) resulting from early-deafness in a visual region (PLLS) of cat cortex. Spines were measured only in neurons that met the criteria of exhibiting a pyramidal morphology and the results are shown in Table 2. A total of 226 neurons were counted and mapped, 577 dendritic segments measured, 17,085 spines counted, and 5,152 spine head diameters measured. Average values for measures of dendritic spine density (measured in spines/micron) by case are shown in Figure 3A, where it is evident that similar values were observed in each case (e.g., values varied within 1 SD of the overall sample mean). Similarly, average values for measures of spine head diameter (unit=microns), are shown by case in Figure 3B. Both figures demonstrate differential effect, especially for measures of spine density. As shown in Figure 3A, all deaf cats exhibited spine density values greater than the group mean, while those from hearing controls were all at or below the group mean. Therefore, from this point forward further analysis of dendritic spine density and then dendritic spine diameter will compare early-deaf and hearing groups.

### Dendritic Spine Density:

When grouped and compared according to treatment, the mean spine density for the deaf cats was  $1.171 \pm 0.295$  spines/micron, while the mean for hearing cats was  $0.984 \pm 0.227$  spines/micron (Figure 4). These results demonstrate that there was a significant increase in dendritic spine density of PLLS pyramidal neurons of early-deaf cats ( $p < 0.0001$ ).

Case Number	Deaf/Hearing status	# PLLS Neurons Counted	# of Dendritic segments	# Spines counted	#Spine diameters measured
147	Deaf	37	82	2,544	1,227
150	Hearing	33	100	3,298	1,191
163	Hearing	42	96	2,862	1,032
164	Deaf	39	116	3,506	1,078
204	Deaf	7	37	1,040	129
211	Hearing	40	86	2,119	321
321	Deaf	28	60	1,716	174
Total Hearing cases		115	282	8,279	2,543
Total Deaf cases		111	295	8,806	2,608
Total ALL		226	577	17,085	5,152

Table 2: Number of PLLS neurons examined, the number of dendritic segments, the number of spines counted, and number of dendritic spines measured for their diameter for each case by its hearing or early-deaf status. It also shows the total results from all the cases for each parameter.

Due to the fact that auditory inputs to visual PLLS differ between the upper third and lower two-thirds segments (Clemo et al. 2008), and because auditory responses differ between the two subregions (Allman et al., 2007), it is expected that plasticity following early-deafness would differentially affect the two areas. This possibility was evaluated by grouping the spine density data according to the upper versus lower PLLS subregions from which it was derived. Figure 5 shows the values for deaf and hearing cats arranged by their upper versus lower PLLS location. Surprisingly, the spine density means for the upper and lower regions for the deaf cats were not significantly different from one another ( $1.142 \pm 0.277$  and  $1.199 \pm 0.309$ , respectively), and the means for the upper and lower regions for the hearing cats also were not significantly different ( $1.001 \pm 0.217$  and  $0.966 \pm 0.237$ , respectively). However, mean spine density values were significantly different between the upper portions of the PLLS in early-deaf versus hearing cats (see Figure 5;  $p < 0.0001$ ) as were the lower portions of the PLLS in these same cases ( $p < 0.0001$ ). These data indicate that early-deafness affects dendritic spine density in the PLLS, but this effect was not based on the differential distribution of auditory inputs or responses in that region.

Since no difference was found between spine density values derived from the upper versus lower portions of the PLLS, data from the two subregions were grouped together for the following analysis. It is well known that the laminar structure of cortex receives differential inputs and executes different processing features (Douglas and Martin, 2004) and that laminar-specific auditory projections to PLLS occur (Clemo et al, 2008). Therefore, spine density data was grouped according to its supragranular (SG) (layers 1-3) or infragranular (IG) (layers 5-6) cortical location and then compared by deaf/hearing status. Figure 6 shows that the spine density in deaf cats' SG layers was  $1.193 \pm 0.298$  spines/micron, while the IG layers was  $1.147 \pm 0.291$

spines/micron which was not a statistically significant difference. Likewise, the spine density in hearing cat SG layers was  $1.002 \pm 0.220$  spines/micron, while for the IG layers was  $0.963 \pm 0.235$  spines/micron which was again not statistically significant. However, as shown in Figure 6, laminar differences in spine density were apparent between PLLS neurons in hearing and early-deaf cats ( $p < 0.0001$ ). These results show that dendritic spine plasticity results from early-deafness, but it is not dependent on or distributed according to cortical lamination.

Another feature that contributes to neuronal processing is the dendritic compartment in which inputs arise. Dendrites are highly branched structures and the level of branching corresponds to the distance of inputs away from the parent cell body (or soma). In order to track branching trends, dendritic spine density was tabulated according to the branch order of the dendritic segment measured, and then hearing and early-deaf conditions were compared (Figure 7). As shown in Figure 7, no particular branch order was preferentially affected by early-deafness and the same trend (deaf density > hearing density) is apparent across the different branch levels. These data demonstrate that deafness-induced dendritic spine plasticity is similarly distributed across the different compartments of PLLS neurons.

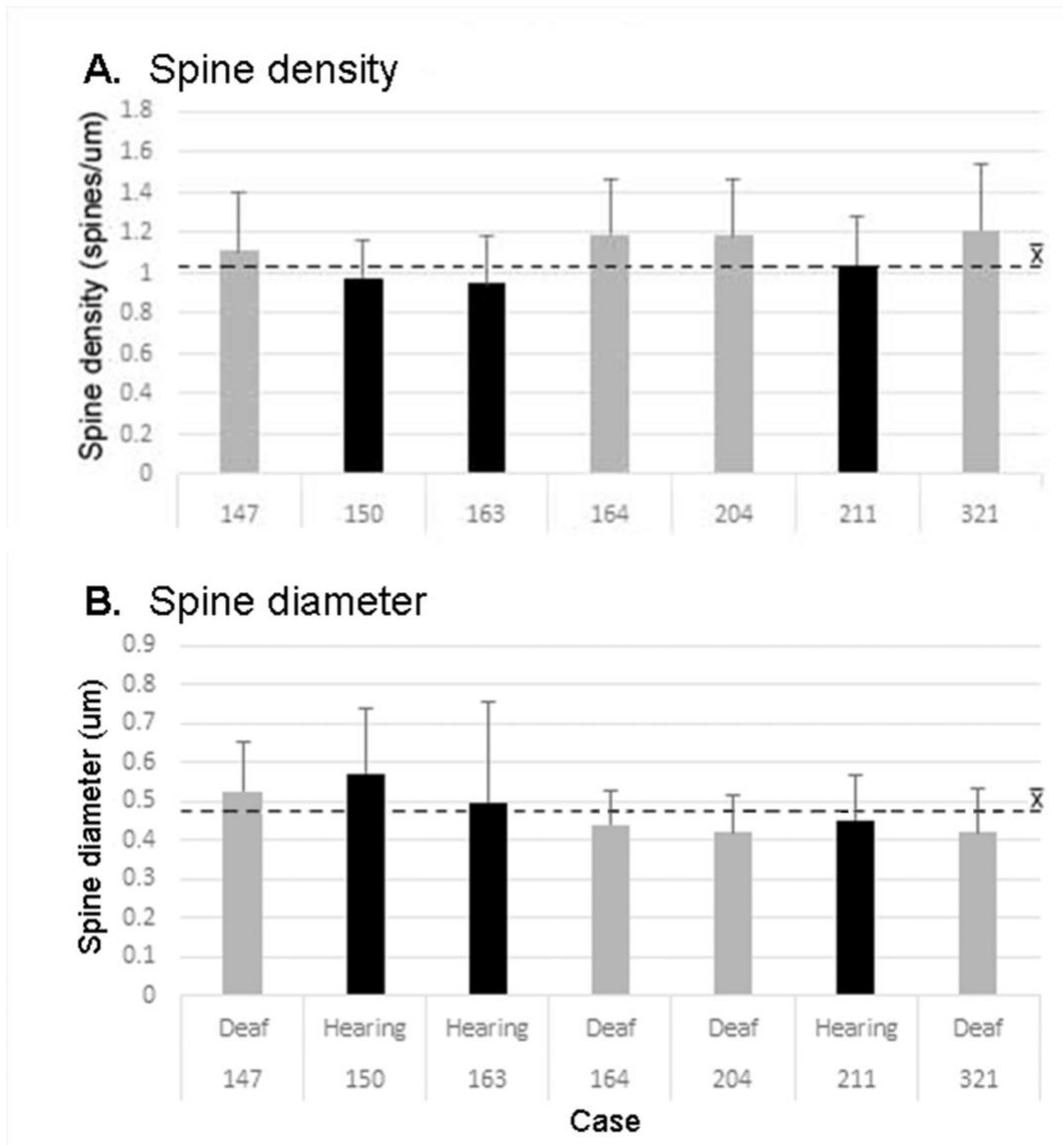


Figure (3): A. Average ( $\pm$  SD) spine density measured from each case, which vary closely around the mean value for all cases. The group mean is denoted by a dashed line marked ' $\bar{x}$ .' Note how all deaf case mean values exceed the group mean while the hearing controls are less than the group mean). B: Average ( $\pm$  SD) spine diameter measured from each case, which vary closely around the mean value for all cases. The group mean is denoted by a dashed line marked ' $\bar{x}$ '. Deaf cases are indicated by grey bars; hearing cases are indicated by black bars.

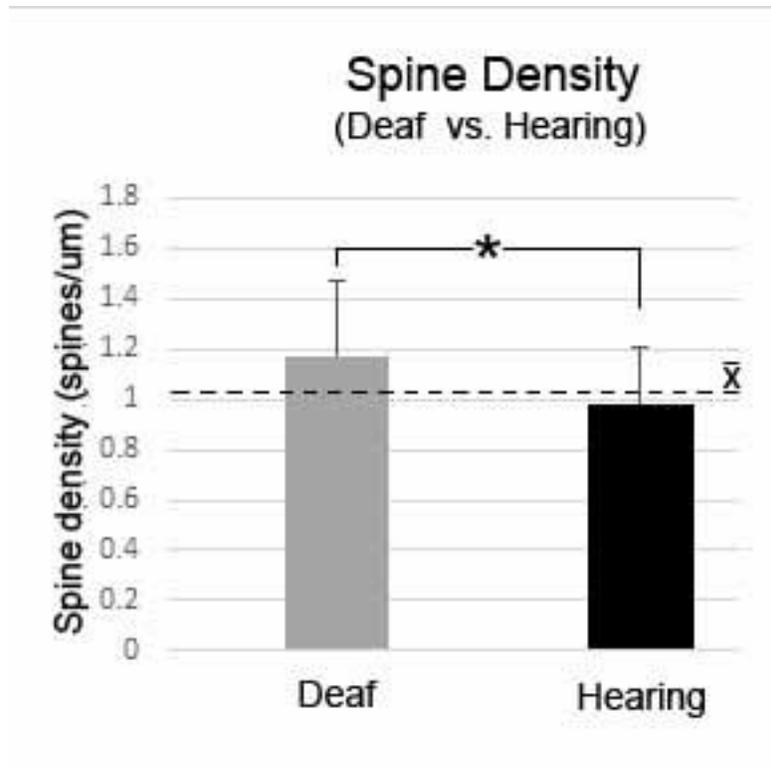


Figure (4): Average ( $\pm$  SD) spine density measured for deaf and hearing cats. Deaf cases are indicated by grey bars; hearing cases are indicated by black bars. The difference in these average values is statistically significant (\*);  $p < 0.0001$ .

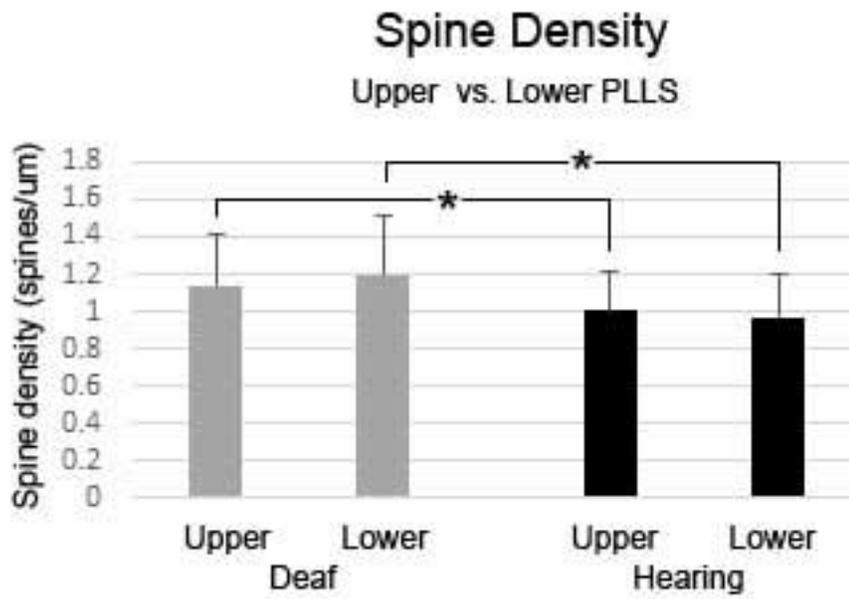


Figure (5): Average ( $\pm$  SD) spine density measured for the upper third region of the PLLS and lower two-thirds region of the PLLS for deaf and hearing cats. Deaf cases are indicated by grey bars; hearing cases are indicated by black bars. Asterisks (\*) indicate that the differences between the deaf and hearing values are statistically significant ( $p < 0.0001$ ).

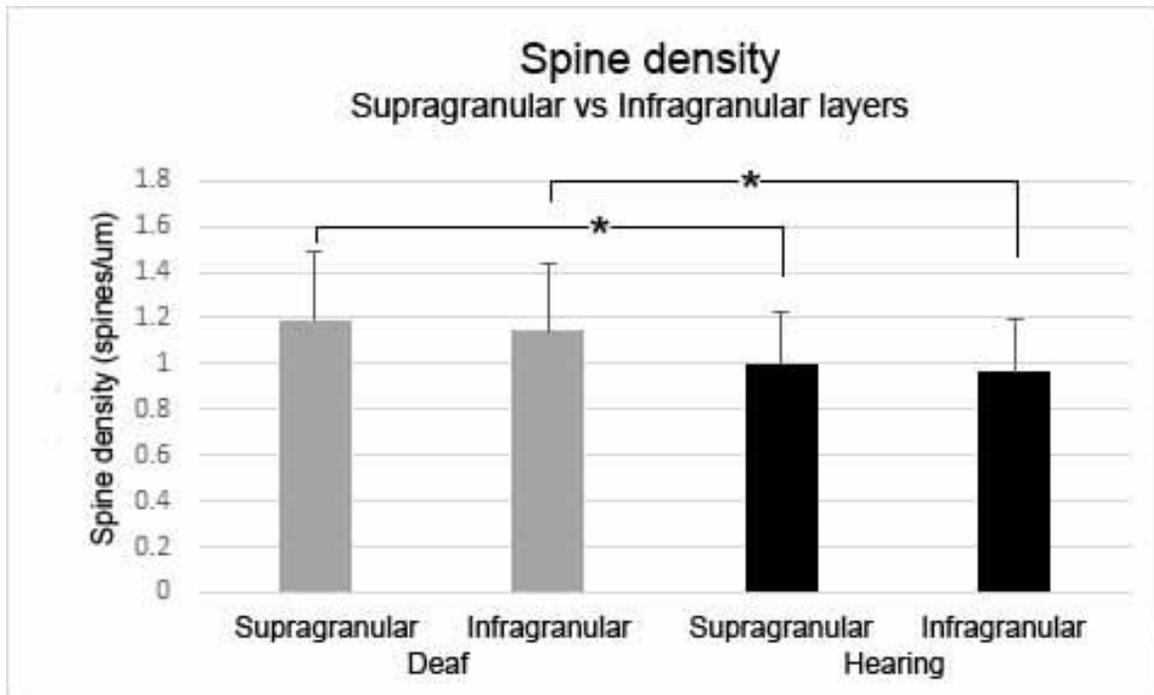


Figure (6): Average ( $\pm$  SD) spine density measured for supragranular (SG) layers and infragranular (IG) layers for deaf and cats. Deaf cases are indicated by grey bars; hearing cases are indicated by black bars. Asterisks (\*) indicate that the differences between the deaf and hearing values are statistically significant ( $p < 0.0001$ ).

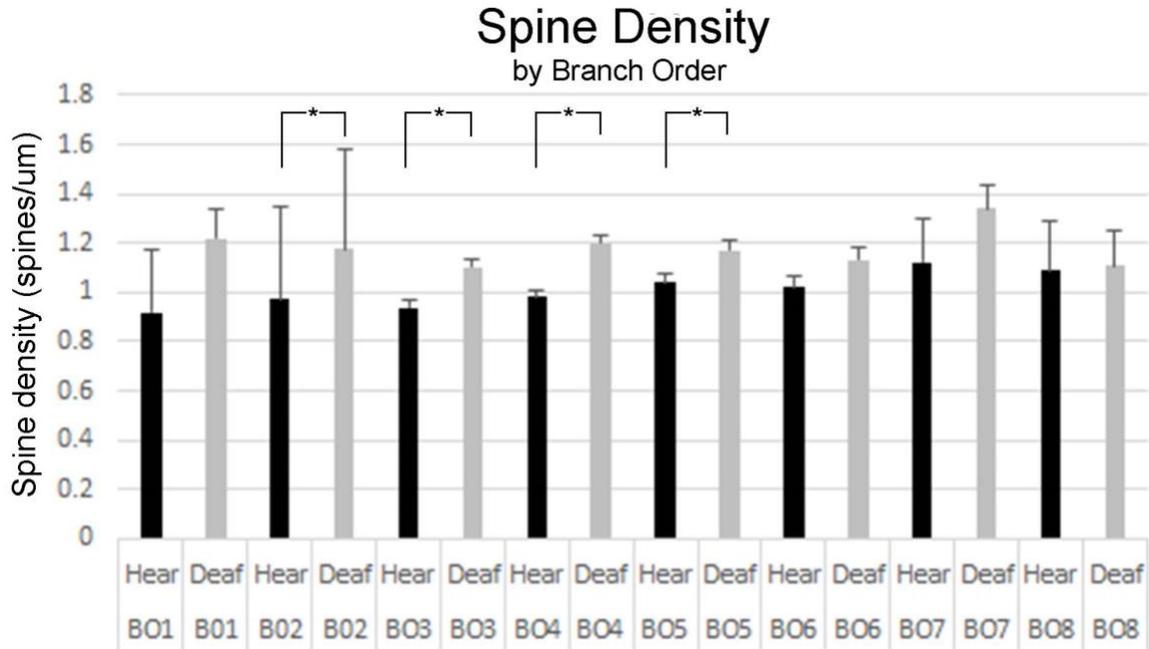


Figure (7): Average ( $\pm$  SD) spine density measured for dendritic segments of Branch Order (BO) 1-8 for deaf and hearing cats. Deaf cases are indicated by grey bars; hearing cases are indicated by black bars. Asterisks (\*) indicate that the differences between the deaf and hearing values are statistically significant ( $p < 0.05$ ).

## Spine Diameter

Spine head diameter is correlated with synaptic maturity and stability (Holtmaat and Svoboda 2009) and this parameter was measured from 5,152 spines on PLLS pyramidal neurons from both hearing and early-deaf cases. When grouped and compared according to treatment, the mean spine diameter for the deaf cats was  $0.478 \pm 0.119$  microns, while the mean for hearing cats was  $0.527 \pm 0.211$  microns. These values, shown in Figure 8, were found to be statistically significant ( $p < 0.0001$ ). These results demonstrate that there was a significant decrease in dendritic spine diameter of PLLS pyramidal neurons of early-deaf cats. However, the difference between the diameters of hearing and deaf cats is extremely small (0.049 microns) while the standard deviation is 2-4 times as large.

As stated earlier, auditory responses differ between the two subregions (Allman et al., 2007), so it might be expected that plasticity following early-deafness would differentially affect the two areas in regards to spine diameter as well. This possibility was evaluated by grouping the spine diameter data according to the upper versus lower PLLS subregions from which it was derived. Figure 9 shows the values for deaf and hearing cats arranged by their upper versus lower PLLS location. The spine diameter means for the upper and lower regions for the deaf cats were small but significantly different from one another ( $p < 0.027$ ) with mean values of  $0.480 \pm 0.113$  and  $0.474 \pm 0.126$ , respectively. On the other hand, the means for the upper and lower regions for the hearing cats were not significantly different ( $0.522 \pm 0.149$  and  $0.531 \pm 0.259$ ), respectively). More important are the comparisons between the early-deaf and hearing cats in the upper and lower regions. It was found that the mean spine diameter values were significantly different between the upper portions of the PLLS in early-deaf versus hearing cats (Figure 9;  $p < 0.0001$ ) as were the lower portions of the PLLS in these same cases ( $p < 0.0001$ ). These data

indicate that early-deafness affects dendritic spine diameter in the PLLS. However, the differences between these values are extremely small.

As stated previously, the laminar structure of cortex receives differential inputs and executes different processing features (Douglas and Martin 2004) and the PLLS primarily receives auditory projections to its supragranular layers (Clemo et al., 2008). Therefore, spine diameter data was grouped according to its supragranular (SG) or infragranular (IG) cortical location and then compared by deaf/hearing status (Figure 10). The spine diameter means for the SG and IG layers in deaf cats were found to be statistically different ( $p < 0.0005$ ) with mean values  $0.485 \pm 0.121$  microns and  $0.469 \pm 0.117$  microns, respectively. Yet, the difference between the SG and IG layers in deaf cats was extremely small (0.016 microns). However, the means for the SG and IG layers in hearing cats were not statistically different ( $0.519 \pm 0.136$  and  $0.536 \pm 0.273$  microns respectively). However, as shown in Figure 10, laminar differences in spine diameter were apparent between PLLS neurons in hearing and early-deaf cats ( $p < 0.0001$ ). This result, like that of measures of spine density, show that dendritic spine plasticity results from early-deafness, but it is not dependent on or distributed according to cortical lamination.

As stated above for spine density measurements, the branch order can provide insight on the neuronal processing of each dendritic compartment. Thus, dendritic spine diameter was organized according to the branch order of the dendritic segment measured, and then hearing and early-deaf conditions were compared. As shown in Figure 11, no particular branch order was preferentially affected by early-deafness since the same trend (hearing diameter  $>$  deaf diameter) is apparent across the different branch levels. These data demonstrate that deafness-induced dendritic spine plasticity is distributed across the different dendritic compartments of PLLS neurons.

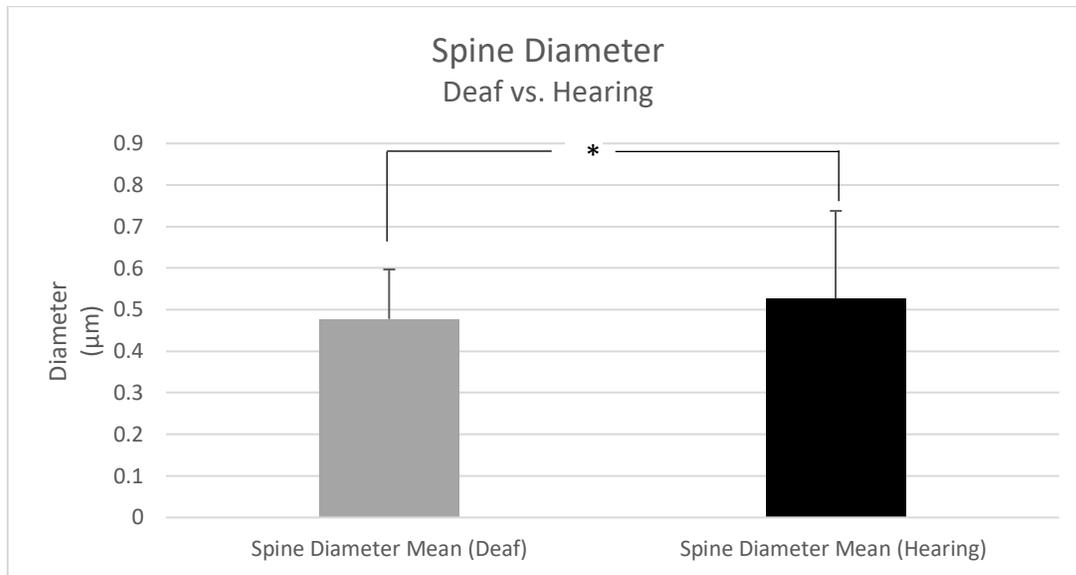


Figure (8): Average ( $\pm$  SD) spine diameter measured for deaf and hearing cats. Deaf cases are indicated by grey bars; hearing cases are indicated by black bars. Asterisk (\*) indicate that the differences between the deaf and hearing values are statistically significant ( $p < 0.0001$ ).

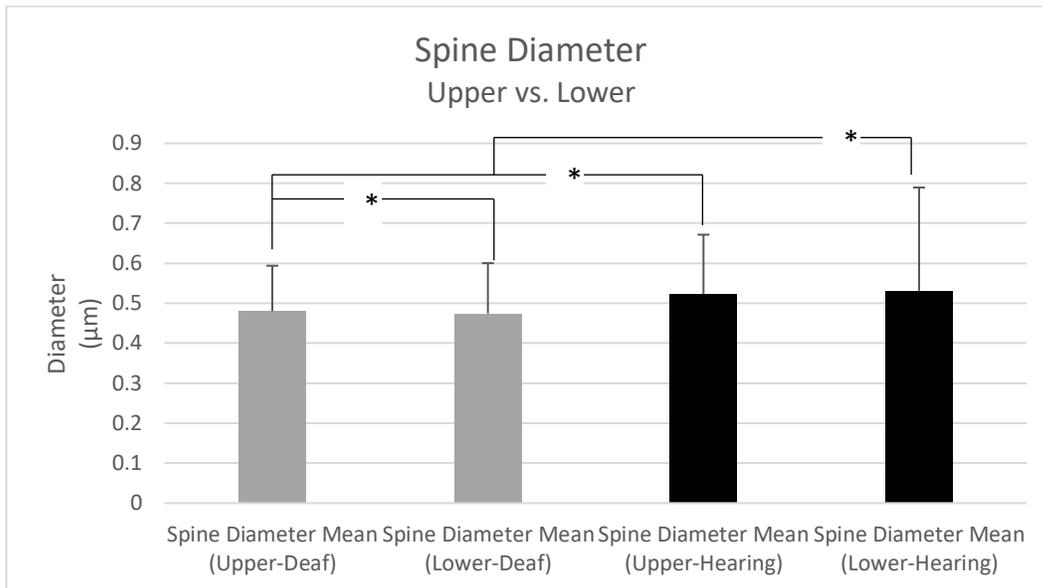


Figure (9): Average ( $\pm$  SD) spine diameter measured for the upper third region and lower two-thirds region of the PLLS for deaf and hearing cats. Deaf cases are indicated by grey bars; hearing cases are indicated by black bars. Asterisks (\*) indicate that the differences between the deaf and hearing values are statistically significant ( $p < 0.05$  or better).

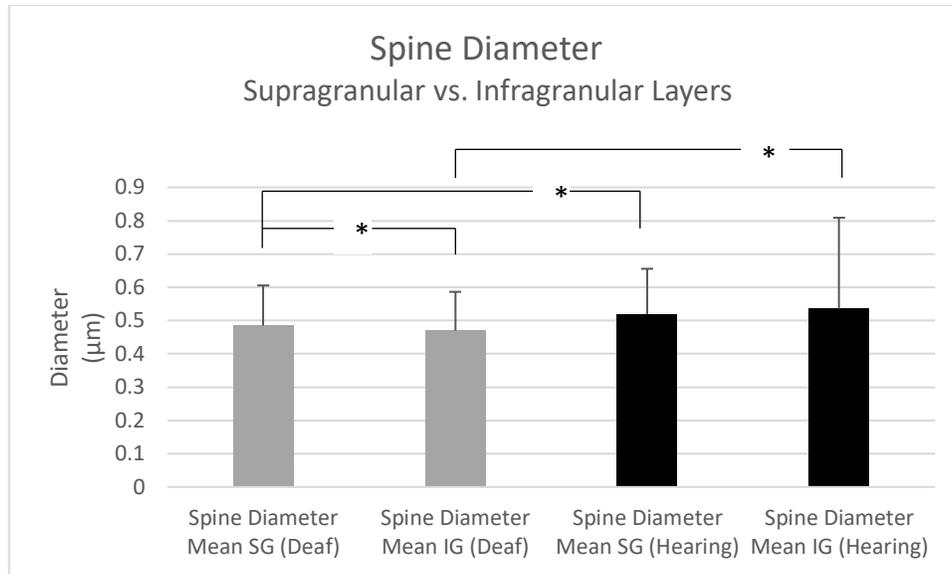


Figure (10): Average ( $\pm$  SD) spine diameter measured for supragranular (SG) layers and infragranular (IG) layers for deaf and hearing cats. Deaf cases are indicated by grey bars; hearing cases are indicated by black bars. Asterisks (\*) indicate that the differences between the deaf and hearing values are statistically significant ( $p < 0.05$  or better).

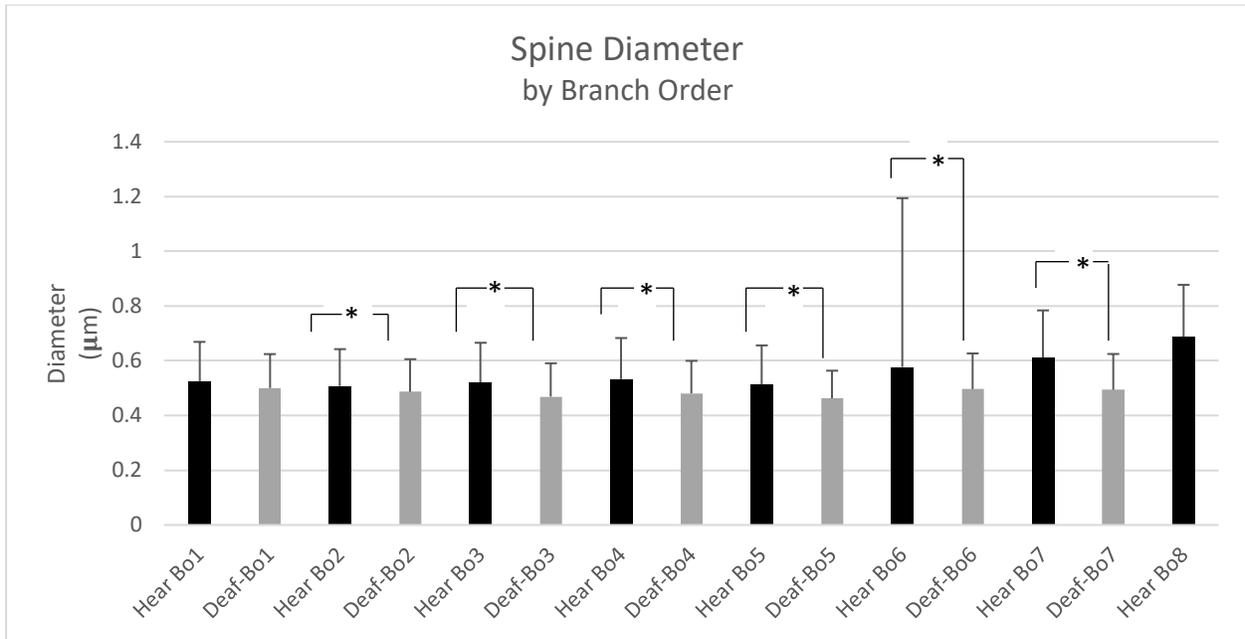


Figure (11): Average ( $\pm$  SD) spine diameter measured for dendritic segments of Branch Order (BO) 1-8 for deaf and hearing cats. Deaf cases are indicated by grey bars; hearing cases are indicated by black bars. Asterisks (\*) indicate that the differences between the deaf and hearing values are statistically significant ( $p < 0.05$  or better).

## Discussion

These results indicate that the dendrites in the visual PLLS region of early deaf cats had a higher spine density than the dendrites in the hearing cats. It was found there was a uniform increase in spine density in both the upper and the lower regions of the PLLS of the early-deaf cats compared to the same regions hearing cats. However, the upper and lower regions of the deaf cats were not significantly different from one another in regard to spine density. Similarly, the early deaf cats had a higher spine density in the supragranular (SG) and infragranular (IG) layers than did the same layers in hearing cats. In light of recent A1 and FAES studies, this project also investigated if early-deafness could cause differences between the supra- and infragranular layers in deaf cats. However, the spine density found in SG and IG layers of the early deaf cats were not significantly different from one another. Finally, there was a higher spine density for every dendritic branch order measured in the early-deaf cats when compared to the hearing cats. Collectively, these data suggest that early-deafness increases dendritic spine density throughout the PLLS region of the visual cortex regardless of location, lamination, or branch order.

Another finding is that measures of dendritic spine diameter were smaller in size in the PLLS region of the visual cortex for the early-deaf cats. There was a uniform decrease in diameter in all regions of the PLLS and all branch orders compared to hearing. Similarly, the spine diameter in the SG and IG layers was smaller in early-deaf cats than hearing cats of the same layer. Finally, the spine diameter was smaller in every branch order that was measured in early-deaf cats compared to hearing cats. However, even though the spine diameter differences were well inside the margin of error, these differences were extremely small. It could be possible

that the statistical test used (t-test) is not as robust when it comes to these extremely small differences and it could be possible that these statistical differences are not as significant or noteworthy.

These generalized changes in PLLS synaptology were in response to loss of auditory signals following early-deafness. Previous studies have found that auditory cortical areas DZ, AI, AII, and the FAES all send projections to the PLLS (Clemo et al. 2008) and have preferred laminar targets into the PLLS. The DZ, AI, and FAES all tended to terminate in the SG layers over IG layers (Clemo et al. 2008). With these projections onto the PLLS, auditory responses in the PLLS would be expected, as was first demonstrated by Yaka et al., (2002). Further studies of the PLLS found that auditory activity showed a differential distribution, where the upper region of the PLLS exhibited bimodal neurons that respond to auditory and visual stimuli, while the lower portion of the PLLS showed only subthreshold auditory influences (Allman and Meredith, 2007). Thus, auditory inputs and activity within the PLLS exhibit a specific distribution pattern of input termination and function.

In order to get an idea of what could possibly happen to PLLS during early-deafness, it was paramount to look at examples of the specificity of distribution of inputs and/or function have been observed in relation to the synaptic changes that occur in auditory cortex after early deafness. For example, a study that examined the effect of early deafness in the FAES region of the auditory cortex observed that spine density in early-deaf cats was significantly higher than the hearing cats; however, this increase happened in the SG layer of the FAES, while the IG showed no such increase (Clemo, Lomber, and Meredith 2016). Within the SG layers in early-deaf cats, the apical dendrites had a statistical increase of spine density (Clemo, Lomber, and Meredith, 2016). Another study that examined A1 showed similar results (Clemo, Lomber, and

Meredith, 2017). The SG layers of the A1 in early-deaf cats had a greater increase in spine density than the IG layers (Clemo, Lomber, and Meredith 2017). However, in the Clemo et al. (2016) study, branch order did not seem to have specificity in early-deaf cats. In fact, it was a general increase of spine density across the branch orders. It could be that in the SG layers of the FAES, there may be other inputs in these neurons that are not auditory in nature. It was found in a previous study that early-deafness in the FAES does not induce new inputs or projections and that visual responsiveness increases (Meredith et al. 2016). This suggests that there were visual inputs already in the SG regions of the FAES. It is possible that these increases in the SG layer in the FAES and A1 are due to the visual inputs in these areas making up for the loss of auditory input, and that these visual inputs in the SG layer were distributed throughout the branch orders. Finally, another study showed the differing results and that early-deafness in the A1 led to a decrease of spine density in layer 3 pyramidal neurons (Macharadze et al. 2019). However, the gerbils in the study that were induced with auditory deprivation in this experiment were deafened on the 10<sup>th</sup> day postnatal and were sacrificed on the 28<sup>th</sup> day postnatal (the end of the gerbil's sensory critical period). In this short time frame cross-modal plasticity may not have occurred yet. It is possible that since the gerbils in the study were sacrificed right at the end of their sensory critical period, their synapses had not yet been given the chance to adapt and change as the cats in the present study who lived an additional three to 32 months before sacrifice and evaluation.

The studies described above indicate that there is a level of specificity that accompanies dendritic spine changes resulting from early deafness. In contrast, the present study shows a general non-specific distribution of dendritic spine changes in the PLLS. That leaves a question of how to resolve this major difference in findings. A possible solution could be that early-

deafness leads to different outcomes in auditory versus visual regions mainly because of the proportion and distribution of the replacement modality. When auditory cortex loses auditory inputs and function, the proportionally small non-auditory inputs that it normally receives must amplify and expand based on the specific locations that they originally accessed the auditory cortex. In contrast, the PLLS is a visual cortex where visual inputs are distributed broadly across its dimensions, so when the sparse auditory inputs are lost due to deafness, synaptic replacement by the visual system can occur at all locations, layers and dendritic levels. The latter condition certainly corresponds with what was observed in the present study.

#### Future Directions:

It has been shown that early deafness results in laminar specificity of spine plasticity in the auditory cortex and that cross-modality plasticity also occurs following blindness (Lewis, Saenz, and Fine 2010; Sadato et al. 1998). Auditory motion discrimination (Saenz et al. 2008; Poirier et al. 2006), localization (Gougoux et al. 2005; Weeks et al. 2000; Leclerc et al. 2000; Collignon et al. 2009; Voss et al. 2004; T. Kujala et al. 1992), pitch change discrimination (T. Kujala et al. 1997; Teija Kujala et al. 2005), and language processing (H. Burton et al. 2002; H. Burton, Diamond, and McDermott 2003; Amedi et al. 2003; Harold Burton and McLaren 2006) are some perceptual effects that are affected by cross-modal plasticity. Therefore, a possible future study could be one that would examine a region of the auditory cortex that also has visual inputs (such as the FAES region), and cause early-induced blindness. It would be important to see if early-blindness induces general (not specific) increases of spine density in the auditory region which would support a general pattern of plasticity suggested by the present study.

## Conclusions:

In conclusion, the present study found that early-deaf cats resulted in an increase in spine density and a decrease in spine head diameter in a visual cortical region in both the upper and lower PLLS regions. Therefore, these findings demonstrate that the visual system showed signs of plasticity from the loss of an auditory stimulus. Ultimately, because PLLS is normally dominated by visual inputs and activity, there is a strong possibility that the new synapses formed in the PLLS in early-deaf cats were visual, suggesting that a cross-modal lesion (early-deafness) can lead to an intramodal change.

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