

Differential Modification of Cortical and Thalamic Projections to Cat Primary Auditory Cortex Following Early- and Late-Onset Deafness

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ABSTRACT

Following sensory deprivation, primary somatosensory and visual cortices undergo crossmodal plasticity, which subserves the remaining modalities. However, controversy remains regarding the neuroplastic potential of primary auditory cortex (A1). To examine this, we identified cortical and thalamic projections to A1 in hearing cats and those with early- and late-onset deafness. Following early deafness, inputs from second auditory cortex (A2) are amplified, whereas the number originating in the dorsal zone (DZ) decreases. In addition, inputs from the dorsal medial geniculate nucleus (dMGN) increase, whereas those from the ventral division (vMGN) are reduced. In late-deaf cats, projections from the anterior auditory field (AAF) are amplified, whereas

those from the DZ decrease. Additionally, in a subset of early- and late-deaf cats, area 17 and the lateral posterior nucleus (LP) of the visual thalamus project concurrently to A1. These results demonstrate that patterns of projections to A1 are modified following deafness, with statistically significant changes occurring within the auditory thalamus and some cortical areas. Moreover, we provide anatomical evidence for small-scale crossmodal changes in projections to A1 that differ between early- and late-onset deaf animals, suggesting that potential crossmodal activation of primary auditory cortex differs depending on the age of deafness onset. *J. Comp. Neurol.* 523:2297–2320, 2015.

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INDEXING TERMS: anatomical connectivity; auditory deprivation; BDA; crossmodal plasticity; hearing loss

In the deaf brain, regions of cortex that would normally respond to sound (auditory areas) 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; Lambert et al., 2005; Pekkola et al., 2005; Lomber et al., 2010; Meredith et al., 2011; Karns et al., 2012). Similarly, in the blind, the occipital cortex, which typically responds to visual stimuli, becomes responsive to auditory (Kujala et al., 1995; Roder et al., 1996, 1999, 2002; De Volder et al., 1997; Weeks et al., 2000) or somatosensory stimuli (Sadato et al., 1996; Cohen et al., 1997; Buchel et al., 1998; Amedi et al., 2002, 2003; Burton et al., 2002). These phenomena are examples of cross-modal plasticity and reflect the

impressive capacity of the brain to adapt to its environment (Bavelier and Neville, 2002). Such reorganization is often functionally beneficial, in that it can lead to enhanced performance in the intact sensory modalities compared with unaffected individuals. For example, people with deafness perform more accurately than hearing subjects in detecting and localizing visual

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stimuli (Reynolds, 1993; Bottari et al., 2011; Scott et al., 2014; Shiell et al., 2014), especially if those stimuli are presented in the visual periphery (Neville et al., 1983). Similarly, individuals with blindness outperform sighted subjects in sound localization tasks (Leclerc et al., 2000; Doucet et al., 2004; Collignon et al., 2006; Fieger et al., 2006). One possible explanation for these behavioral advantages following sensory deprivation involves a greater cortical volume devoted to the remaining senses; however, the ability of the brain to rearrange cortical inputs appears to depend on when sensory deprivation occurs (Hensch, 2004; Sharma et al., 2005, 2009). For example, when presented with a moving visual stimulus, the extent of activation in visual cortex is greater in prelingually deaf subjects than in postlingually deaf subjects (Buckley and Tobey, 2011). Thus, the degree of functional reorganization appears to be greater following early-onset hearing loss than following late-onset hearing loss; this pattern is in clear accordance with conventional views of how neuroplastic potential declines with age. Although residual neural plasticity likely underlies behavioral changes in the case of late-onset sensory deprivation (Kujala et al., 1997; Buchel et al., 1998; Ponton et al., 2001; Allman et al., 2009; Kok et al., 2013), studies of blind or deaf individuals reveal that the location or structure that is reorganized may differ between early- and late-onset sensory deprivation (Cohen et al., 1999; Sadato et al., 2002; Sadato, 2006; Chabot et al., 2007).

In various species, primary sensory areas undergo reorganization following the loss of a modality. For example, within the somatosensory system, inputs to primary cortical areas are subject to reorganization following the complete deafferentation of their primary

input (Qi et al., 2011; Bowes et al., 2012, 2013). Moreover, primary visual areas undergo crossmodal reorganization in an age-dependent manner, with greater activation in the occipital cortex by haptic stimuli in humans with early-onset blindness than in those with late-onset blindness (Buchel et al., 1998; Cohen et al., 1999; Sadato et al., 2002; Wittenberg et al., 2004). In contrast, there remains some controversy about the potential of the primary auditory cortex (A1) to reorganize crossmodally following deafness (Lambertz et al., 2005). An early study in cats found A1 to be visually responsive if deafness occurred during the first postnatal week, but not if deafness was induced at 2 months of age (Rebillard et al., 1977). However, subsequent studies failed to find evidence of cross-modal reorganization in A1 of congenitally deaf cats (Stewart and Starr, 1970; Kral et al., 2003). Although these functional studies appear somewhat contradictory, a recent anatomical study shows clear evidence of a significant reduction in size of A1 in early- and late-deaf cats (Wong et al., 2013).

Because there is a reduction in the size of A1 in deaf cats, a change in the global connectivity of A1 following deafness was expected. However, because few (Rebillard et al., 1977; Finney et al., 2001) or no responses to heteromodal stimuli (Stewart and Starr, 1970; Kral et al., 2003; Mayberry et al., 2011; Leonard et al., 2012) have been reported, it remained unclear what the nature of these changes would be. Moreover, the level of the auditory pathway at which these changes may take place is also unknown. Seminal studies in adults suggested that intramodal reorganization of tonotopic maps occurs at a cortical level (Robertson and Irvine, 1989; Rajan et al., 1993). However, the degradation of tonotopic maps in A1 following early-onset, partial damage of the auditory sensory epithelium demonstrates that the patterns of projections from the auditory thalamus are preserved (Stanton and Harrison, 2000). Moreover, studies of crossmodal plasticity in higher order auditory areas have suggested that reorganization may reflect changes at a distant, subcortical nucleus (Allman et al., 2009), whereas retrograde labeling of thalamic projections to primary sensory areas indicates that crossmodal connections that might subserve multisensory plasticity persist into adulthood (Henschke et al., 2014). Thus, the goal of this study was to describe how thalamocortical and corticocortical projections to A1, including those from other modalities, are altered by the onset of deafness (Fig. 1).

MATERIALS AND METHODS

A total of 15 adult domestic cats were examined. They were obtained from a USDA licensed commercial

Abbreviations

| | |
|--------|--------------------------------------------------------|
| A1 | Primary auditory cortex |
| A2 | Second auditory cortex |
| AAF | Anterior auditory field |
| ABR | Auditory brainstem response |
| ALLS | Anterolateral lateral suprasylvian area |
| BDA | Biotinylated dextran amine |
| CO | Cytochrome oxidase |
| dB nHL | Decibels normal hearing level |
| dPE | Dorsal posterior ectosylvian gyrus |
| DZ | Dorsal zone of auditory cortex |
| EEG | Electroencephalography |
| Epp | Posterior ectosylvian field |
| FAES | Auditory field of the anterior ectosylvian sulcus |
| iPE | Intermediate division of the dorsal ectosylvian cortex |
| LGnd | Dorsal division of the lateral geniculate nucleus |
| LP | Lateral posterior nucleus |
| MGB | Medial geniculate body |
| MGBd | Dorsal division of the medial geniculate body |
| MGBm | Medial division of the medial geniculate body |
| MGBv | Ventral division of the medial geniculate body |
| PAF | Posterior auditory field |
| PLLS | Posterolateral lateral suprasylvian area |
| V1 | Primary visual cortex |
| VP | Ventral posterior nucleus |

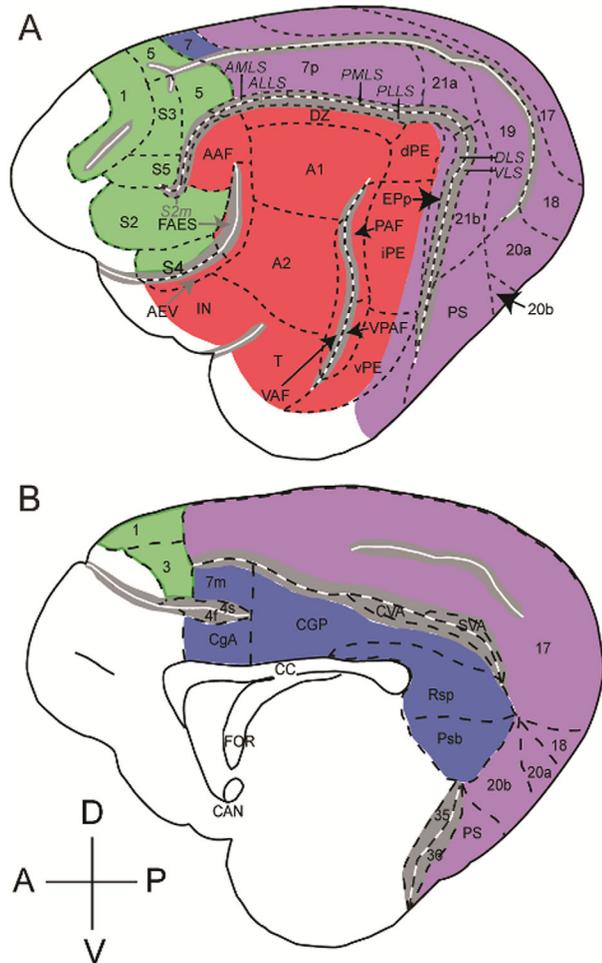


Figure 1. Schematic lateral (A) and medial (B) views of the cat brain. The auditory (red), visual (purple), somatomotor (green), and other (blue) cortical areas analyzed in this study are highlighted. The bottom of each sulcus is represented by a white line and the cortex in the bank is gray. Dashed lines indicate cortical area borders. Dorsoventral and anteroposterior axes are indicated at bottom left. For abbreviations, see list.

breeding facility (Liberty Laboratories, Waverly, NY), and were divided into three groups: five early-deaf animals, with hearing loss onset postnatally around the time of hearing onset (<3 weeks); five late-deaf animals, with hearing loss onset at no earlier than 4 months of age; and five normal hearing animals (Fig. 2). The early-deaf animals provide a model in which auditory input is removed within the critical period for normal development of auditory function, when the potential for the reorganization of neural connections is greatest. Late-deaf animals received normal auditory input during development, only to have this input removed following the closure of this period of maximal plasticity.

In all cases, deafness was confirmed by the absence of stimulus-evoked activity in the auditory brainstem

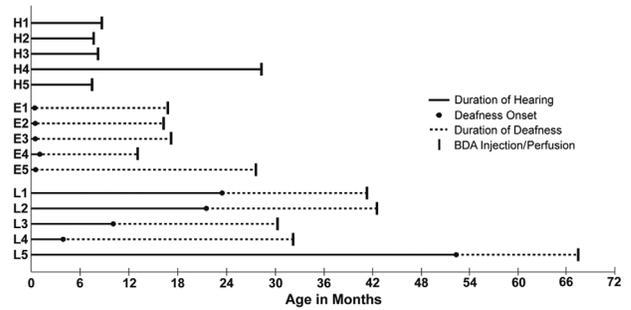


Figure 2. Experimental timeline for the 15 cats examined following early- or late-onset deafness. The duration of hearing is illustrated by solid horizontal lines and deafness onset is indicated by solid black circles. The duration of deafness is represented by horizontal dashed lines. After a period of deafness lasting at least 11 months, or after at least 8 months of age in hearing animals, retrograde BDA injections were made in A1 and animals were perfused (vertical lines). The age at onset of deafness (early vs. late) was significantly different ($t(8) = -2.587$, $P = 0.032$), whereas the duration of deafness was not ($t(8) = -0.911$, $P = 0.39$).

response (ABR; Fig. 3). All surgical and experimental procedures were conducted in accordance with the Canadian Council on Animal Care's *Guide to the Care and Use of Experimental Animals* (Olfert, 1993) and were approved by the University of Western Ontario Animal Use Subcommittee of the University Council on Animal Care.

Onset of deafness

The five cats that constituted the early-deaf group had auditory thresholds shifted ototoxically around the time of hearing onset (<3 weeks postnatal; Shipley et al., 1980), and the five late-deaf cats underwent an identical procedure at no earlier than 4 months of age. In all cases, hearing loss was induced through the coadministration of kanamycin and Edecrin® (ethacrynic acid; Valeant Pharmaceuticals, Laval, Quebec). This method is known to cause cochlear hair cell destruction and a subsequent permanent, profound hearing loss (Xu et al., 1993). Prior to the procedure, animals were anesthetized by spontaneous inhalation of oxygen (1 L/min) and isoflurane (5% to effect for induction, then 1.5–2% to maintain). Baseline hearing thresholds (Fig. 3A) were assessed using ABRs elicited by auditory stimuli (0.1-ms squarewave clicks; range 0–80 dB nHL) presented through ER3A foam insert headphones (Etymotic Research, Elk Grove Village, IL). Electroencephalography (EEG) leads were inserted subdermally above the right and left ears, with a reference lead placed at the vertex, and a ground placed at the lower back.

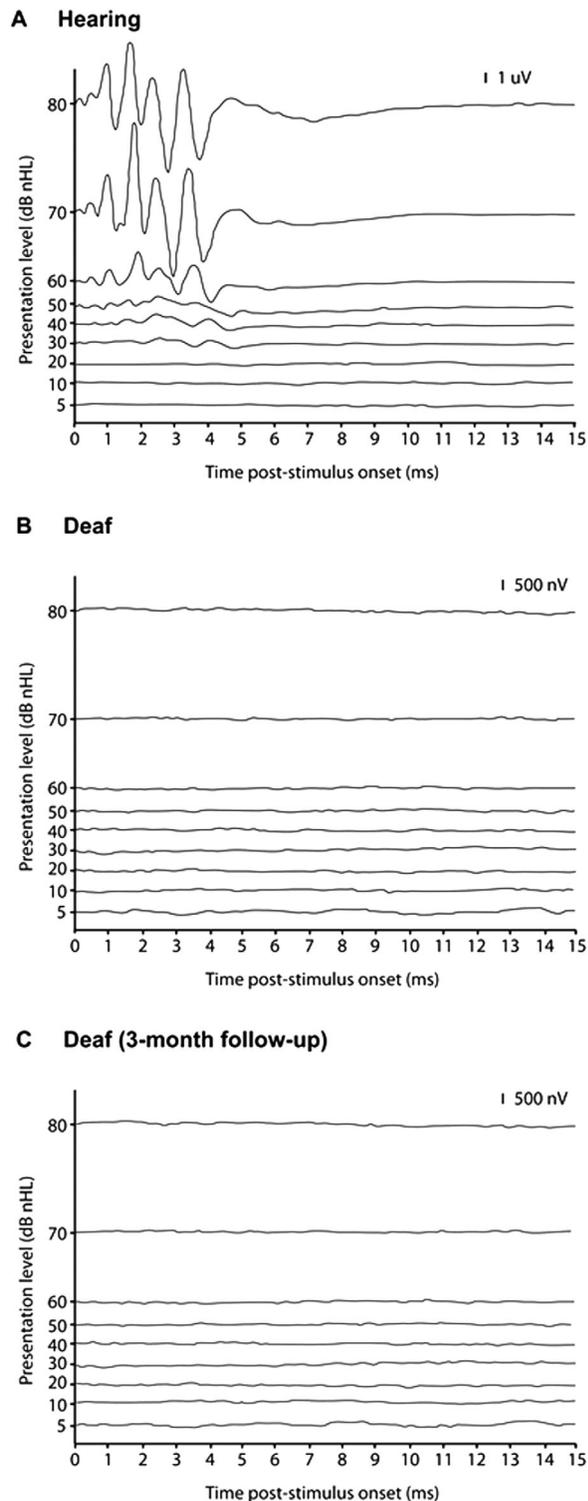


Figure 3. Auditory brainstem responses to click stimuli of increasing sound intensities up to 80 dB nHL. **A:** In the hearing cat, typical responses were observed with peaks that decreased in amplitude and increased in latency with decreasing stimulus intensity. **B:** Representative ABR from a cat (E5) immediately after deafness onset. **C:** 3-month post-deafness onset follow-up ABR from the same animal as in B.

An intravenous catheter was inserted into either the cephalic vein of the forelimb, or the jugular vein at the neck. Animals were injected with kanamycin (300 mg/kg, s.c.) and infused with ethacrynic acid (to effect: 35–60 mg/kg, i.v.). ABRs were collected continuously until responses were absent at all presentation levels (Fig. 3B). Once auditory thresholds were elevated beyond 80 dB nHL, the infusion of ethacrynic acid was stopped, and replaced with lactated Ringer's solution (4 ml/kg, i.v.). Following this, the catheter was removed and the animals recovered from the anesthetic. Follow-up ABRs were performed 3 months post deafness onset to confirm that the procedures were successful (Fig. 3C).

Tracer deposits

The afternoon prior to the surgical procedure, the cats were fasted and lightly anesthetized with ketamine (4 mg/kg, i.m.) and Domitor (0.05 mg/kg, i.m. medetomidine hydrochloride; Pfizer Animal Health, Exton, PA). An indwelling feline catheter was inserted into the cephalic vein to permit administration of anesthetic during surgery. The catheter was flushed with heparinized saline, and an anti-inflammatory medication was given (dexamethasone, 0.05 mg/kg, i.m.). On the day of surgery, each cat was given atropine (0.02 mg/kg, s.c.) to minimize respiratory and alimentary secretions, acepromazine (0.02 mg/kg, s.c.), an additional dose of dexamethasone (0.5 mg/kg, i.v.), and buprenorphine (0.01 mg/kg, s.c.). Sodium pentobarbital (25 mg/kg to effect, i.v.) was administered to induce general anesthesia. The topical anesthetic Cetecaine was sprayed onto the pharyngeal walls to inhibit the gag reflex, and the cats were intubated. The head was shaved and stabilized in a stereotaxic apparatus. The cats were then prepared for surgery using antiseptic procedures. Respiration rate, blood pressure, and heart rate were monitored throughout the procedure, and body temperature was maintained at 37°C by using a water-filled heating pad (Gaymar, Orchard Park, NY).

A midline incision was made, and the left temporalis muscle was reflected laterally. A craniotomy was made that extended from the posterior ectosylvian sulcus to the anterior ectosylvian sulcus, and from the suprasylvian sulcus to the lateral border of A1. The bone piece was removed and the dura was reflected laterally.

Biotinylated dextran amine (BDA; 3000 MW, [10%], Vector, Burlingame, CA, Cat# SP-1140, RRI-D:AB_2336249) was pressure-injected (Nanoliter 2000, World Precision Instruments, Sarasota, FL) through a glass pipette. Although it does provide some anterograde labeling, BDA3k is primarily a dependable and robust retrograde pathway tracer (Reiner et al., 2000) that has been shown to be more sensitive than

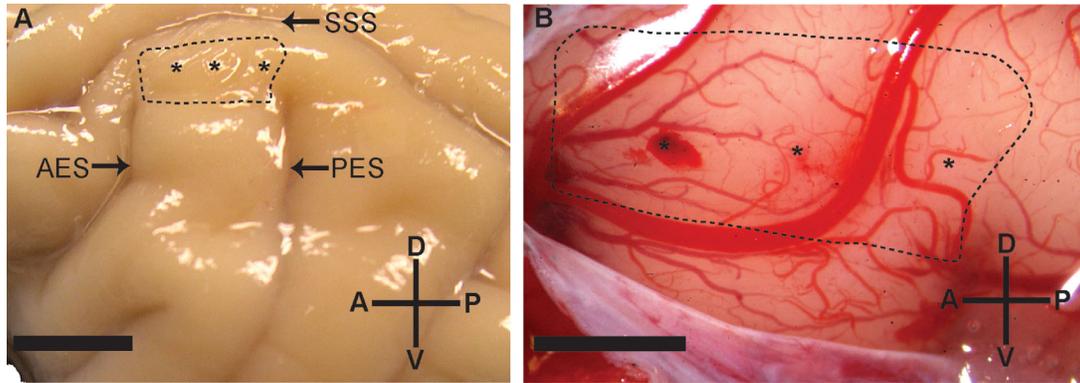


Figure 4. Injection locations in A1. **A:** Lateral view of the cerebrum post perfusion (E5). The asterisks indicate the locations of the three penetrations. **B:** Enlargement of the exposed left A1 from the same animal following craniotomy and BDA injection. The perimeter of A1 is noted by a black dashed line. AES, anterior ectosylvian sulcus; PES, posterior ectosylvian sulcus; SSS, suprasylvian sulcus. Scale bar = 5 mm in A; 2 mm in B.

horseradish peroxidase (HRP), and to label afferent projections more reliably than biocytin or neurobiotin (Lapper and Bolam, 1991). Because A1 occupies a large area of cortex and our goal was to label a substantial subsection of this area, injections were made at three pipette penetrations spanning the left A1. To ensure that injections were made into A1 of all cats, the pipette was placed along a plane lying dorsal to the dorsal tips of the posterior and the anterior ectosylvian sulci. This ensured that the injection was located at the center of A1 in all animals, despite a dorsal shift in the ventral border of A1 in deaf cats (Wong et al., 2013). At each penetration, two deposits were made: 150 nl at a depth of 500 μm from the cortical surface, and an additional 150 nl at a depth of 1,200 μm from the cortical surface, to target superficial and deeper cortical layers, respectively. Three minutes passed after each injection before the pipette was moved. When all three penetrations (six injections total) were completed, the brain was photographed to provide a record of the location of injection sites (Fig. 4), and the craniotomy was closed using dental acrylic anchored to stainless steel skull screws. Once the acrylic had hardened, no further sodium pentobarbital was administered. Instead, cats were given the inhalation anesthetic isoflurane (1.5%) until the incision was closed. Lidocaine was subcutaneously injected around the incision margin.

Postsurgical procedures

Cats were extubated once the swallowing reflex returned. The indwelling catheter was removed, and a bolus of lactated Ringer's solution was given (s.c.) as necessary. Respiration rate, blood pressure, and heart rate were continually monitored until the animal was sternally recumbent. Buprenorphine (0.01 mg/kg, s.c.)

was administered every 6 hours for the first 24 hours after surgery, and every 12 hours for the subsequent 72 hours. Animals also received dexamethasone every 24 hours after the surgery (0.05 mg/kg on day 1, decreasing by 0.01 mg/kg each day thereafter). In all cases, recovery was uneventful.

Perfusion and tissue processing

Two weeks following tracer deposit, the cephalic vein was cannulated, and each animal was deeply anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The anticoagulant heparin (10,000 U; 1 cc) and the vasodilator 1% sodium nitrite (1 cc) were administered. Each animal was then perfused intracardially through the ascending aorta with 1 liter of physiological saline, followed by 2 liters of fixative solution (4% paraformaldehyde), and 2 liters of 10% sucrose solution to cryoprotect the tissue. Each solution was buffered to a pH of 7.4 with 0.1 M Sorenson's buffer and infused at a rate of 100 ml/min. Following the perfusion, the head was mounted in a stereotaxic frame where the brain was exposed and blocked in the coronal plane at Horsley-Clarke (Horsley and Clarke, 1908) level A27. Each brain was then removed from the cranium, photographed, and immersed in a 30% sucrose solution until it sank to cryoprotect it for histological processing.

Brains were frozen and serial sections were cut in the coronal plane at 60 μm using a cryostat Leica CM 3050s (Leica Microsystems, Nussloch, Germany). A total of six series of sections at 360- μm intervals were collected. One series was immunohistochemically processed to reveal the presence of BDA using the avidin-biotin peroxidase method (Covance, Princeton, NJ, Cat# SMI-32R-100, RRID:AB_10123643; Covance, Cat# SMI-5010C-2000, RRID:AB_10120127), with nickel-cobalt

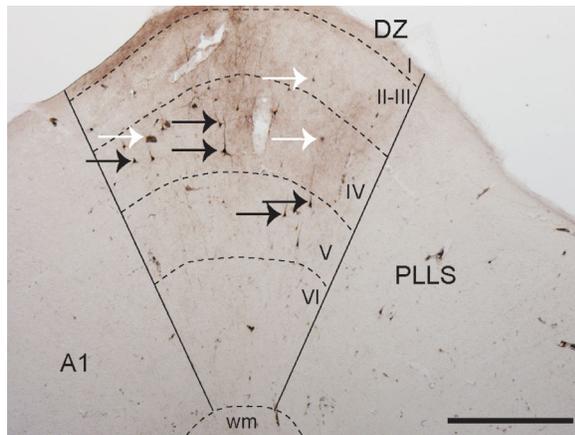


Figure 5. Labeled neurons in the auditory cortex (dorsal zone). The black arrows indicate labeled neurons. In order for a neuron to be labeled, the nucleus had to be visible and the entirety of the somatic membrane had to be present. The white arrows point to neurons that are too faintly labeled and were not included in the count. For abbreviations, see list. Scale bar = 100 μ m.

intensification (Veenman et al., 1992). Three of the remaining series were processed using the monoclonal antibody SMI-32 (Sternberger Monoclonals, Lutherville, MD, Cat# SMI-32, RRID:AB_2315331; Sternberger and Sternberger, 1983), cytochrome oxidase (Payne and Lomber, 1996), or cresyl violet stain to label the Nissl bodies to assist with laminar and other border distinctions. The remaining two series were retained as spares and were processed using the above methods as necessary. All tissue was mounted onto gelatin-coated slides, air-dried, cleared, and coverslipped.

Data analysis

BDA-labeled neurons were visualized using a Nikon E600 microscope equipped with Nomarski DIC imaging and mounted with a DXM 1200 digital camera. Tissue outlines, injection sites, and labeled neurons were plotted by using a PC-driven motorized stage controlled NeuroLucida software (NeuroLucida, RRID:nif-0000-10294). The section contours and the injection site were drawn, and to prevent incomplete sampling, a meander scan was performed to quantify neurons. Using the criteria of Kok and colleagues (2014), neurons were considered labeled only if the entirety of the soma was visible; partial cell bodies or dendritic branches alone were not considered labeled to exclude artifacts of the reaction process, and thus provided a conservative estimate of neuronal projections (Fig. 5). Neurons within the injection site or within the lateral extent of the injection were not counted, to avoid the inclusion of artifactual labeling. Focal levels throughout the z-plane of each section were taken to ensure that

the full thickness was examined. It is important to note that retrograde labeling with BDA has been shown to be stable with age (Rajakumar et al., 1993), such that differences between late-deaf and early-deaf/normal hearing animals could not be attributed to the age of the animal at injection/perfusion.

Labeled neurons were assigned to cortical areas on an individual-animal basis based on cytoarchitecture and sulcal and gyral landmarks defining areal borders. The affinity of SMI-32 for a dephosphorylated epitope on the medium- and high-molecular weight subunits of neurofilament proteins results in robust labeling of pyramidal cells and dendrites, particularly in layers III and V (Mellott et al. 2010). Additionally, patterns of SMI-32 labeling differ by area in auditory and visual cortex, allowing for demarcation of areal borders (van der Gucht et al., 2001; Mellott et al., 2010). Importantly, these staining profiles have been shown to be conserved following hearing loss (Wong et al., 2015). Borders between auditory and somatosensory areas can be distinguished by a marked increase in SMI-32 reactivity (van der Gucht et al., 2001), whereas borders between somatosensory areas are primarily delineated using Nissl labeling profiles (Clascá et al., 1997). Borders between the posterior lateral suprasylvian areas (PLLS and PMLS), and the dorsal and ventral lateral suprasylvian areas (DLS and VLS) of visual cortex were placed on the lateral bank of the middle suprasylvian sulcus and the anterior bank of the posterior limb of the suprasylvian sulcus, respectively (as per Palmer et al. [1978], Updyke [1986], and Rauschecker et al. [1987]). This convention is supported by cytoarchitectonic methods in the visual system (van der Gucht et al., 2001). Labeled neurons determined to lie on the border between two cortical areas, or within the transitional zone between two areas, were distributed equally to each of the two areas. Finally, labeling profiles were constructed for each of the three groups examined, and these groups were contrasted to determine whether any significant differences in connectivity existed. For illustrative purposes, labeled neurons from one animal in each group were plotted on standardized slices to facilitate direct comparison (with labeled neurons repositioned to lie within the correct cortical areas; Figs. 8, 10, 12); neurons from a second animal from each group were plotted on sections from that individual (Figs. 7, 9, 11).

RESULTS

Injection sites and tracer spread

Fifteen cats received deposits of retrograde BDA to ensure tracer uptake in axon terminals throughout all six cortical layers of the left A1. The three injection tracts were circumscribed to an area located between

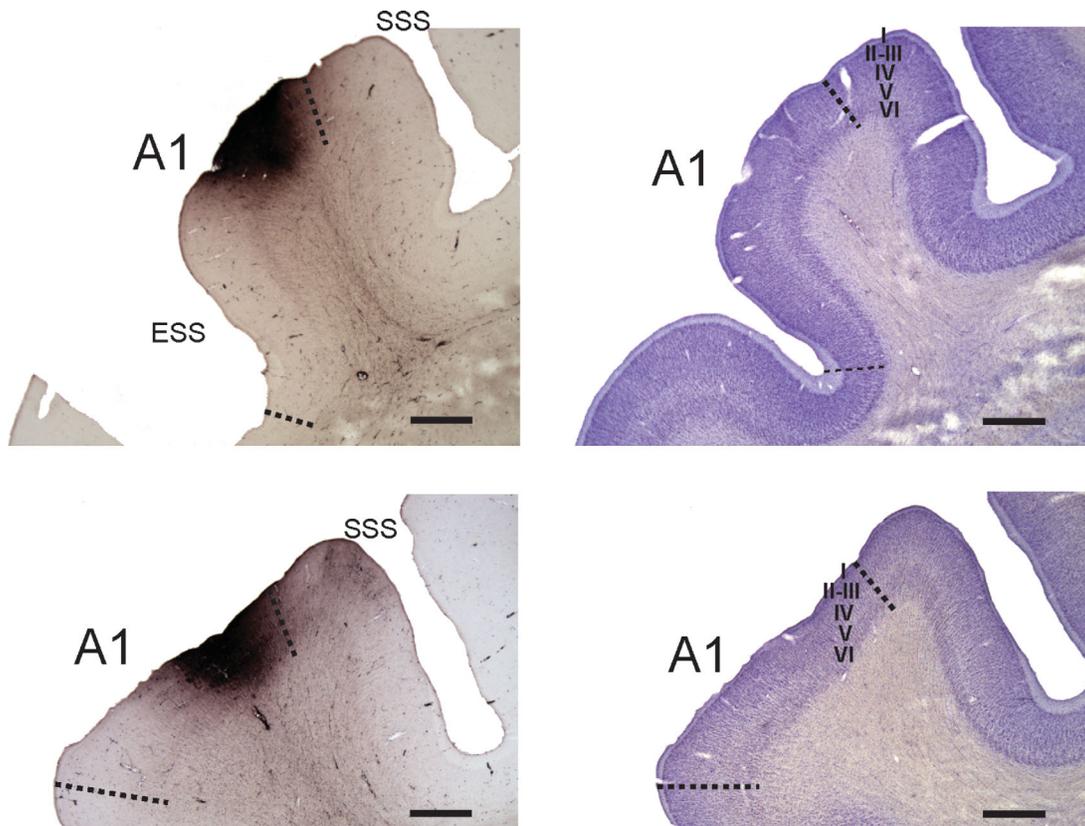


Figure 6. Photomicrographs of coronal sections through A1 showing representative injection sites (L5). The top and bottom rows show the posterior and anterior A1 injections, respectively. The left column shows the BDA injection site and the right column is an adjacent section stained with Nissl. The spread of the tracer was limited to the boundaries of A1, while spanning all six layers of the neocortex. SMI-32 (not shown), Nissl, and CO (not shown), were used to identify the boundaries of cortical areas. A1: primary auditory cortex, ESS: ectosylvian sulcus, SSS: suprasylvian sulcus. Scale bar = 1 mm in all panels.

the anterior and posterior ectosylvian sulci, along a plane dorsal to their most dorsal limit. In all cases, the tracer spread throughout all six cortical layers, with no evidence of tracer deposits in any other cortical area. One representative case is illustrated in Figure 6. However, even with the amount of tracer per injection held constant, and with similar spread, injections in the current study did not permeate A1 to an equal extent in every group. In hearing cats, only the central portion of A1 was filled, whereas the deaf cats received tracer injections encompassing both central A1 and the ventral portion of A1 near its border with the second auditory cortex (A2). This difference is due to the dorsal shift of the A1 ventral boundary in deaf cats (Wong et al., 2013).

Summary of labeled projections to A1

Following a tracer injection in A1, the majority of retrogradely labeled neurons in hearing cats were located in the dorsal portion of the brain, with the ventral portion being sparsely labeled. Labeled neurons were observed in 10 of the auditory areas, namely, the sec-

ond auditory cortex (A2), the anterior auditory field (AAF), the auditory field of the anterior ectosylvian sulcus (FAES), the dorsoposterior ectosylvian gyrus (dPE), the dorsal zone of the auditory cortex (DZ), the intermediate division of the posterior ectosylvian auditory cortex (iPE), the posterior auditory field (PAF), the ventroposterior auditory field, and the ventral portion of the posterior ectosylvian auditory cortex (vPE). In the visual areas, labeled neurons were observed in the anterolateral and the posterolateral lateral suprasylvian (ALLS and PLLS, respectively) areas, and the posterior ectosylvian field (EPp). In somatomotor cortex, labeled neurons were confined to secondary areas. In addition, the claustrum and the caudate also showed labeled neurons after an injection in A1. At the thalamic level, projections to A1 were observed from the three nuclei of the medial geniculate body (MGB). The ventral division of the medial geniculate (MGBv) had the strongest projection, whereas the medial (MGBm) and dorsal nuclei (MGBd) had smaller projections to A1. Thalamic labeling was exclusively ipsilateral (Figs. 7, 8).

Normal Hearing (H5)

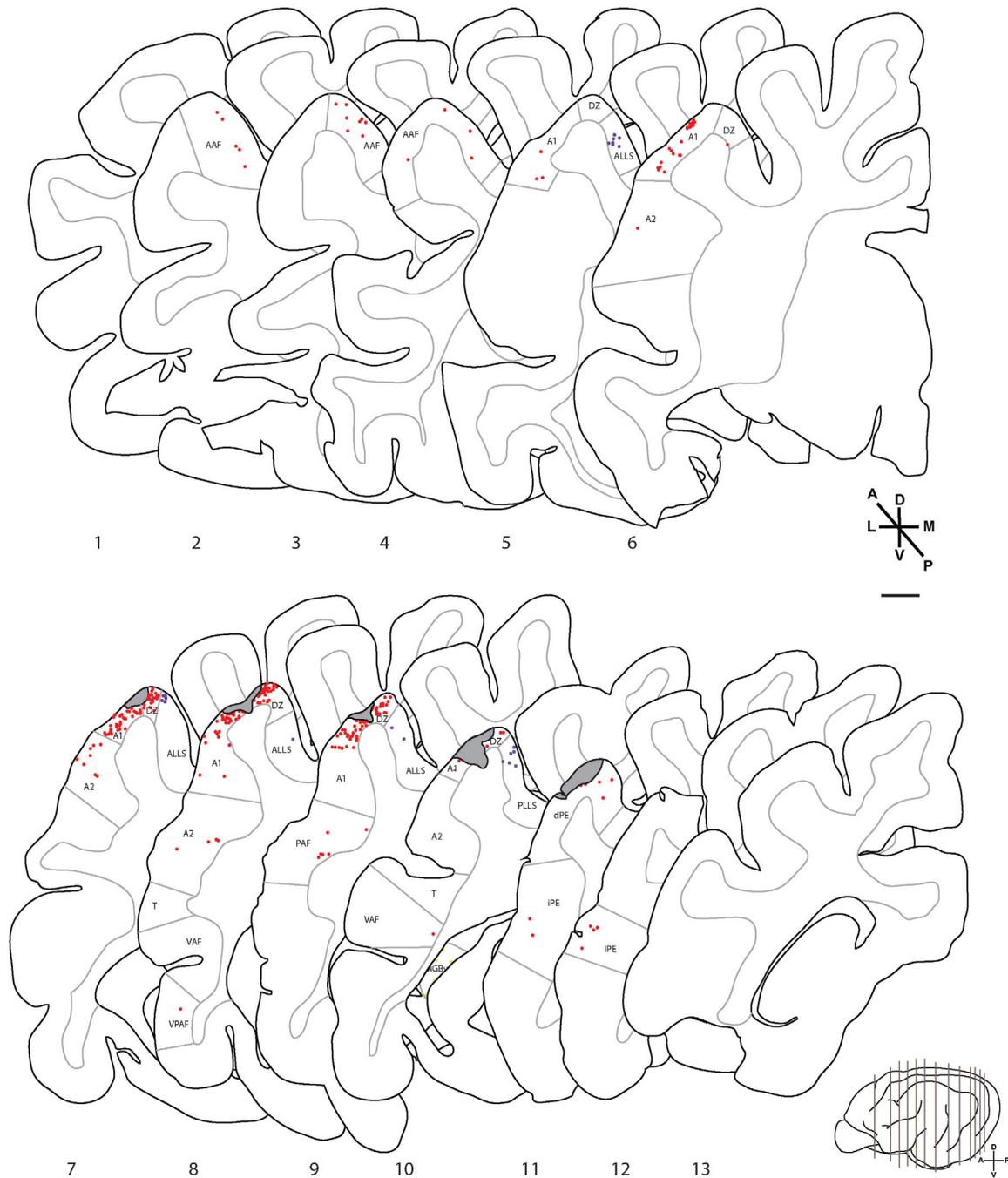


Figure 7. Plots of labeled neurons on coronal sections from a hearing cat (H5), with areal boundaries delimited by SMI-32 labeling profiles in adjacent sections. Injection sites are represented by shading; no neurons were counted within these regions. Color-coded dots represent labeled neurons from auditory cortex (red), visual cortex (purple), and auditory thalamus (yellow). Bottom right: a lateral brain view showing the selected levels from which coronal sections were taken. For abbreviations, see list. Scale bar = 2 mm.

Generally, the areas labeled from A1 were similar across groups. Small increases in the proportion of labeled cells were observed across the majority of auditory cortical areas following hearing loss, whereas a decrease in projections from area DZ was noted in both

early- and late-deaf animals. In addition, some reorganization in inputs from thalamic nuclei was observed between groups. Representative labeling profiles of early-deaf cats are presented in Figures 9 and 10, and Figures 11 and 12 show representative late-deaf cats.

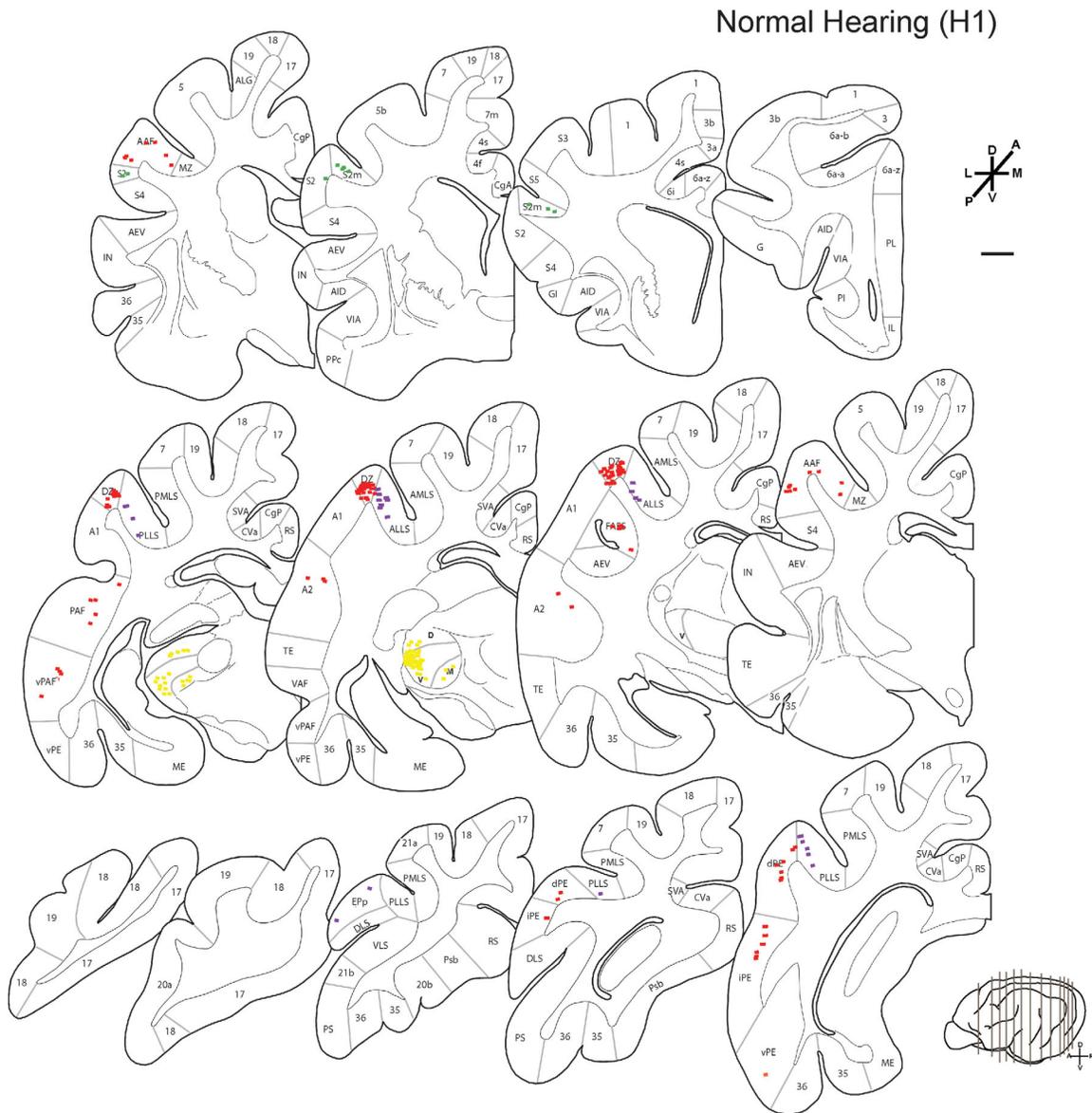


Figure 8. Representative distribution of ipsilateral projections to the primary auditory field of a hearing cat (H1). Color-coded dots represent labeled neurons from auditory (red), visual (purple), somatomotor (green), and other (blue) cortical areas, as well as projections from auditory thalamus (yellow). Bottom right: a lateral brain view showing the selected levels from which the mapped coronal sections were taken. For abbreviations, see list. Scale bar = 2 mm.

Novel, yet inconsistent intermodal projections

In this study, the labeled areas in deaf brains were generally the same as those labeled in the brains of hearing cats. However, two areas provide exceptions. In two early-deaf (E3 and E4) and two late-deaf (L2 and L5) cats, labeled neurons were observed both in area 17 of primary visual cortex (ranging from 0.03% to 0.4% of total labeled neurons; Fig. 10), and in the lateral posterior (LP) nucleus of the thalamus (0.07% to 1% of total labeled neurons; Fig. 11). One additional early-deaf animal (E1) had projections from area 17 alone (0.07% of

total labeled neurons). No animal presented with projections from LP in isolation, and no hearing animal had inputs from either area 17 or the LP nucleus.

Examining projections by modality of origin

All neurons in the brain showing positive labeling were counted. Across all three experimental groups, the relative proportion of connections arising from each cortical and thalamic area containing labeled neurons was determined by dividing the number of labeled neurons in that area by the total number of labeled neurons in the entire brain. Relative proportions of labeled

Early Deaf (E5)

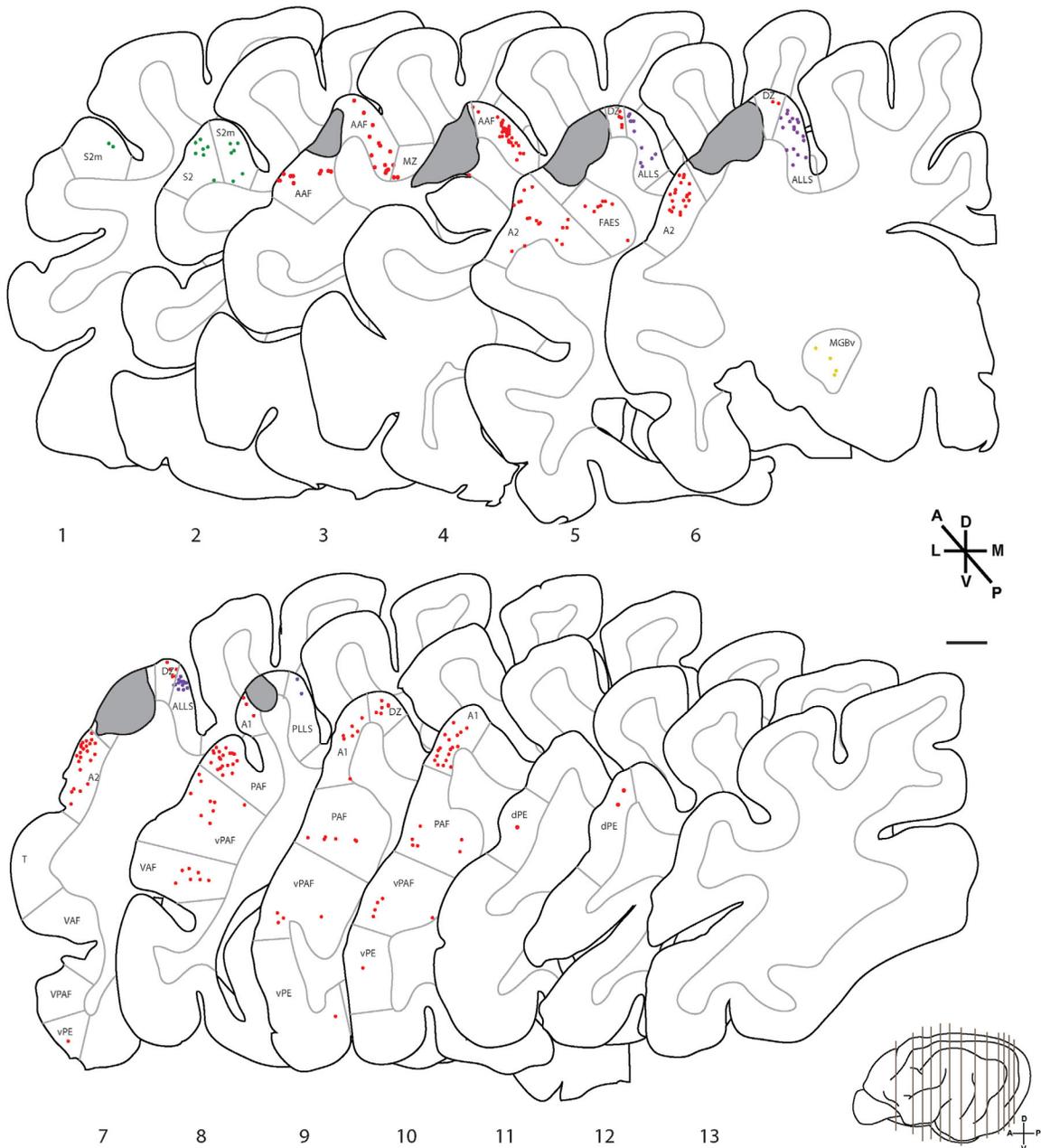


Figure 9. Plots of labeled neurons on coronal sections from an early-deaf cat (E5), with areal boundaries delimited by SMI-32 labeling profiles in adjacent sections. Injection sites are represented by shading; no neurons were counted within these regions. Color-coded dots represent labeled neurons from auditory cortex (red), visual cortex (purple), somatomotor cortex (green), and auditory thalamus (yellow). Bottom right: a lateral brain view showing the selected levels from which coronal sections were taken. For abbreviations, see list. Scale bar = 2 mm.

neurons were used (as opposed to the absolute number of labeled neurons in a given area) to allow meaningful conclusions to be made despite variability in the uptake and spread of BDA, as well as in the immunohistochemical processes used to visualize labeled neurons. To generate group level summaries, projections to A1 were classified as either thalamic in origin, or as arising from

cortical areas typically involved in auditory, visual, somatomotor, or other functions (as per Fig. 1). The total number of projections from each modality was divided by the total number of labeled cells in each group to generate the pie charts in Figure 13. This figure reveals that in both early- and late-onset deafness, the proportion of projections to A1 originating from

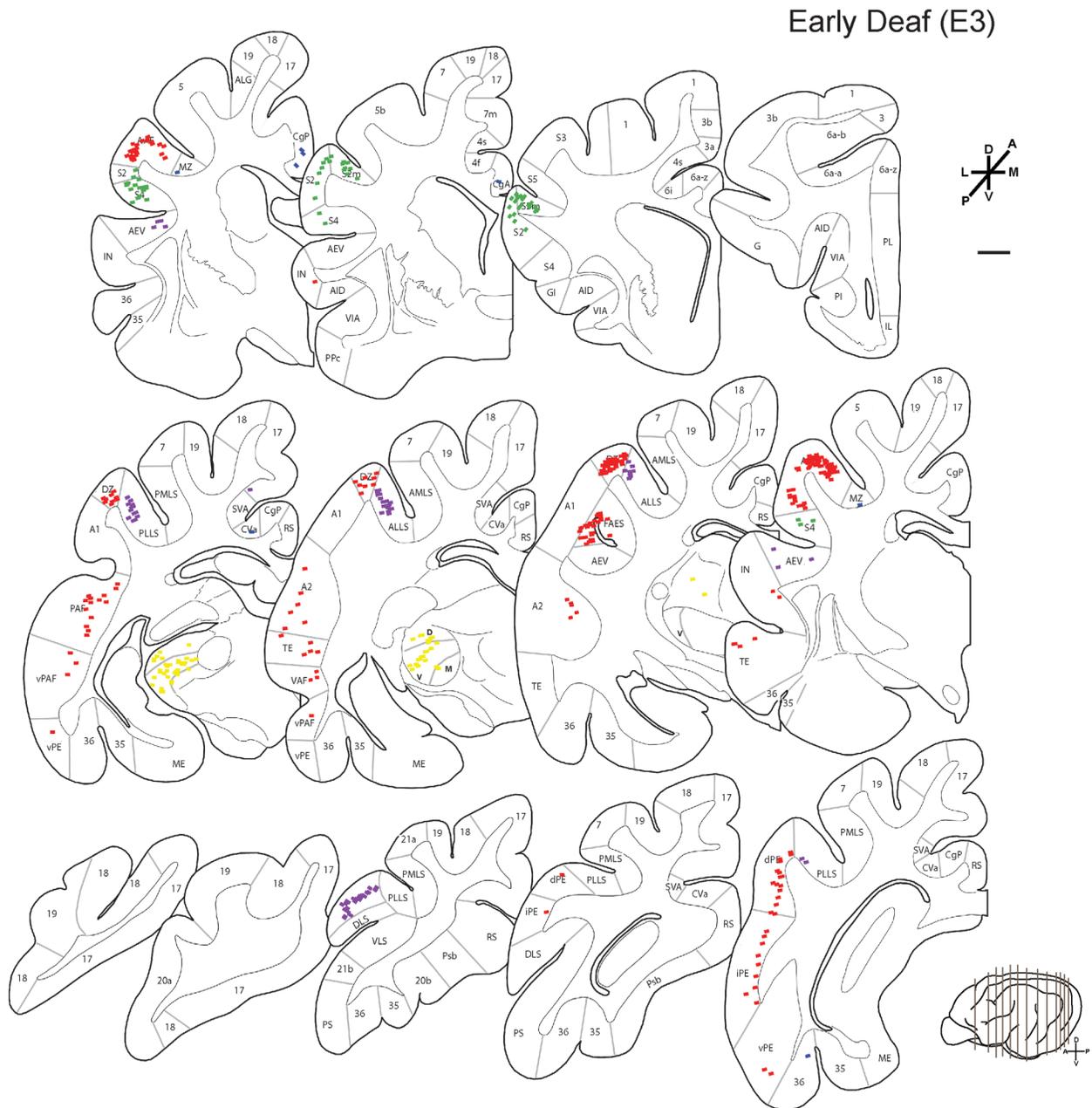


Figure 10. Representative distribution of ipsilateral projections to the primary auditory field of an early-deaf cat (E3). Color-coded dots represent labeled neurons from auditory (red), visual (purple), somatomotor (green), and other (blue) cortical areas, as well as projections from auditory thalamus (yellow). Bottom right: a lateral brain view showing the selected levels from which the mapped coronal sections were taken. For abbreviations, see list. Scale bar = 2 mm.

auditory areas was at a similar level (hearing: 46.45%, early-deaf: 49.61%, late-deaf: 49.60%). In contrast, projections originating from the visual areas were less apparent in the late-deaf animals (hearing: 10.82%, early-deaf: 9.83%, late-deaf: 6.36%), whereas somatomotor projections were more prominent in all deaf animals (hearing: 0.68%, early-deaf: 4.77%, late-deaf: 3.82%). In sum, there was a trend toward modality-level changes in crossmodal projections to A1 following hear-

ing loss that may depend on the age of deafness onset.

Examining group-level differences in projections by area of origin

To investigate the differences in cortical connectivity between hearing and deaf brains, as well as any differences in the level of crossmodal plasticity observed between cats that acquired hearing loss early versus

Late-Deaf (L5)

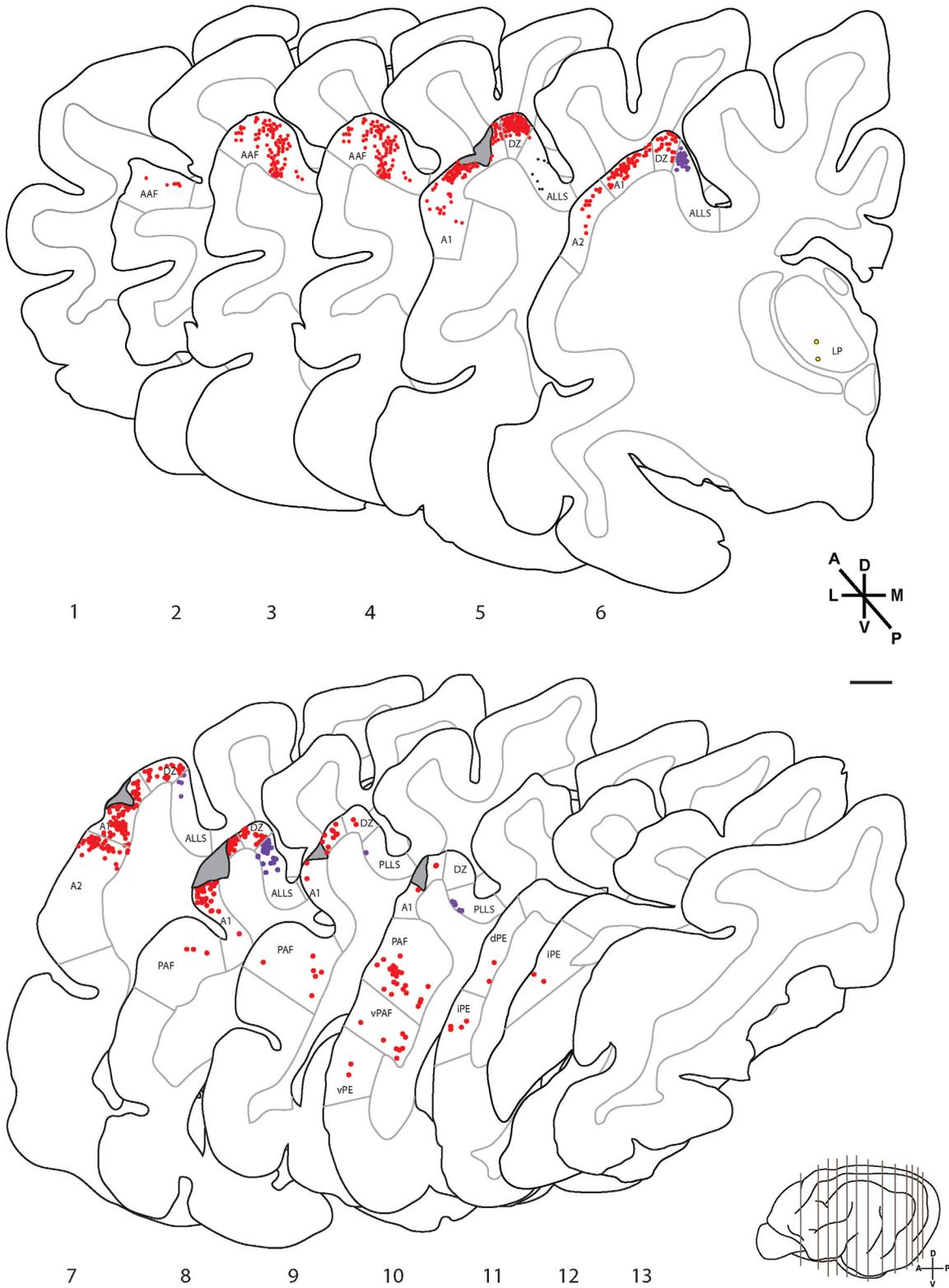


Figure 11. Plots of labeled neurons on coronal sections from a late-deaf cat (L5), with areal boundaries delimited by SMI-32 labeling profiles in adjacent sections. Injection sites are represented by shading; no neurons were counted within these regions. Color-coded dots represent labeled neurons from auditory cortex (red), visual cortex (purple), and auditory thalamus (yellow). Bottom right: a lateral brain view showing the selected levels from which coronal sections were taken. For abbreviations, see list. Scale bar = 2 mm.

Cortical and subcortical projections to A1

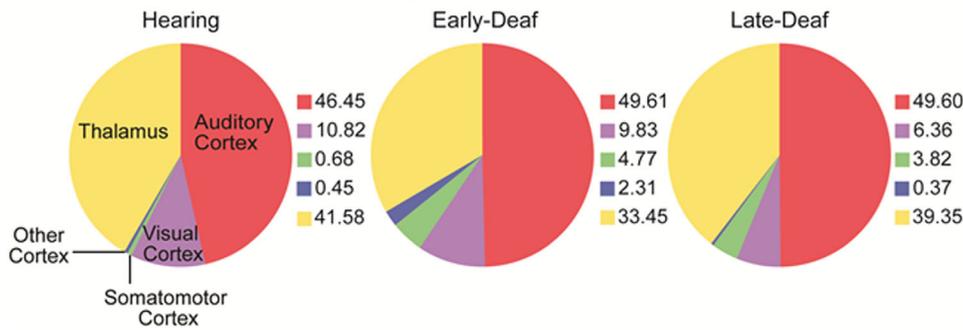


Figure 13. Pie charts displaying the relative percentage of projections to A1 arising from each sensory area of the brain. The percentages of projections arising from each region are indicated to the right of each graph.

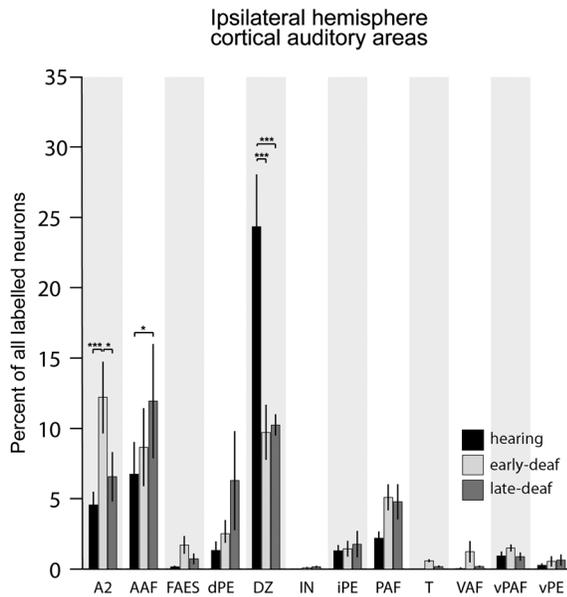


Figure 14. Histogram illustrating the distribution of corticocortical projections from areas in the ipsilateral auditory cortex. The y-axis represents the percent of all labeled neurons. Ipsilateral auditory areas projecting to A1 are listed along the x-axis. Error bars show the standard error of the mean, and significant differences are noted with asterisks (*, $P \leq 0.05$; ***, $P \leq 0.001$). Both early- and late-onset deafness result in fewer projections from DZ to A1, whereas there are significant increases in the number of projections from A2 in early-deaf cats, and from AAF in late-deaf cats. For abbreviations, see list.

DZ (hearing: 24.34%, early-deaf: 9.71%, and late-deaf: 10.24%) were reduced in both early- ($P < 0.001$) and late-deaf animals ($P = 0.001$).

No significant differences between groups emerged in the proportion of labeled projections from visual cortical areas (Fig. 15). Anecdotally, however, there was an interesting pattern of projections from EPP, with an

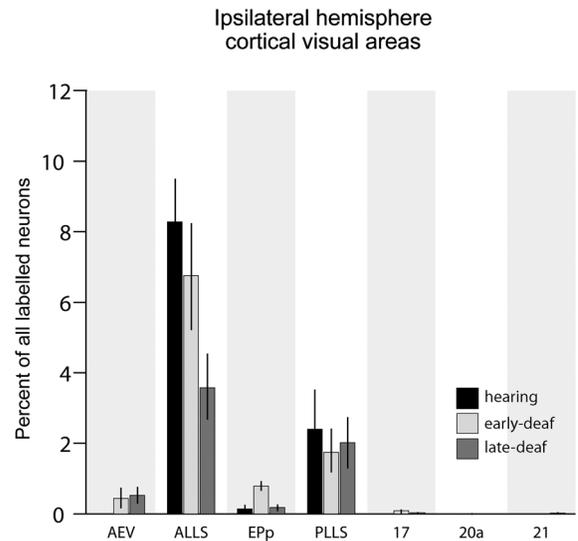


Figure 15. Histogram illustrating the distribution of corticocortical projections from areas in ipsilateral, visual-related cortex. The y-axis represents the percent of all labeled neurons. Ipsilateral visual areas projecting to A1 are listed along the x-axis. Error bars show the standard deviation of the mean, and significant differences are noted with asterisks (***, $P \leq 0.001$). An ANOVA does not reveal a significant change in the strength of projections to A1. For abbreviations, see list.

increase in the proportion of labeled cells for the early-deaf cats only. Although this difference did not reach statistical significance at a group level, a regression analysis considering age at onset of deafness and labeling in EPP revealed a strong significant correlation best fit with a quadratic function (Fig. 16).

Projections to A1 arising from ipsilateral somatomotor cortex, and other ipsilateral telencephalic areas are illustrated in Figures 17 and 18, respectively. No group differences in the strength of projections to A1 from

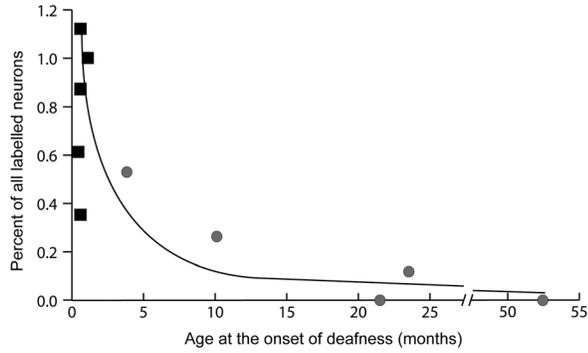


Figure 16. Correlation between age at onset of deafness (x-axis) and labeling of EPP (y-axis). Black squares and gray circles represent individual early-deaf and late-deaf cats, respectively. Note that the early-deaf cats show greater labeling in EPP than late-deaf cats.

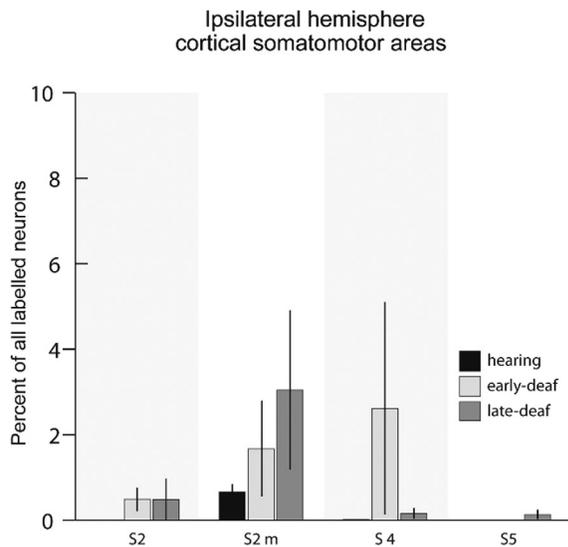


Figure 17. Histogram illustrating the distribution of corticocortical projections from areas in the ipsilateral somatomotor cortex. The y-axis represents the percent of all labeled neurons. Ipsilateral somatomotor areas projecting to A1 are listed along the x-axis. Error bars show the standard deviation of the mean. Note that the ANOVA did not reveal a significant change in the strength of projection to A1 originating from the somatomotor areas.

somatomotor or other cortical areas reached significance.

Thalamic projections to A1 are illustrated in Figure 19. The pattern of projections from the subnuclei of the MGB was the same for all three groups tested. The highest proportion of projections originated in the ventral nucleus (MGBv; hearing: 36.95%, early-deaf: 17.80%, late-deaf: 30.87%), with fewer originating in the dorsal nucleus (MGBd; hearing: 3.61%, early-deaf: 10.83%, late-deaf: 5.56%), and the fewest originating in

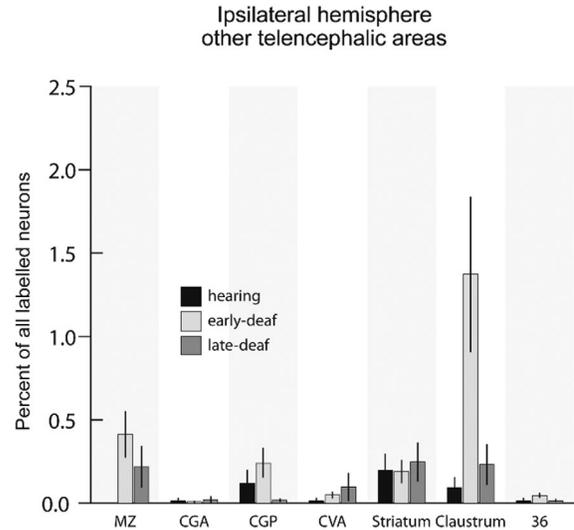


Figure 18. Histogram illustrating the distribution of projections from other ipsilateral telencephalic areas. The y-axis represents the percent of all labeled neurons. Ipsilateral areas projecting to A1 are listed along the x-axis. Error bars show the standard deviation of the mean. Note that the ANOVA did not reveal a significant change in the strength of projections to A1 originating from the other cortices. For abbreviations, see list.

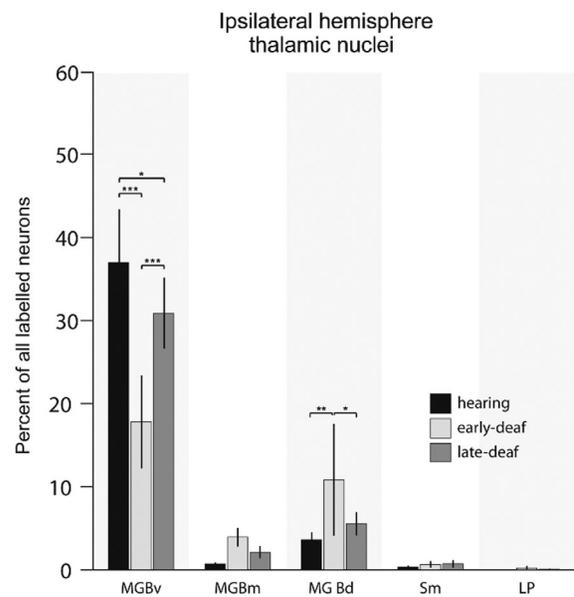


Figure 19. Histogram illustrating the ipsilateral distribution of thalamocortical projections. The y-axis represents the percent of all labeled neurons. Ipsilateral thalamic areas projecting to A1 are listed along the x-axis. Error bars show the standard deviation of the mean, and significant differences are noted with asterisks (*, $P \leq 0.05$; **, $P \leq 0.0005$; ***, $P \leq 0.001$). Note that the ventral part of the medial geniculate body (MGB) shows a significant decrease in early-deaf as well as in late-deaf cats in comparison with hearing cats. The early-deaf cats also show a significant increase in the number of projections from the dorsal part of the MGB. For abbreviations, see list.

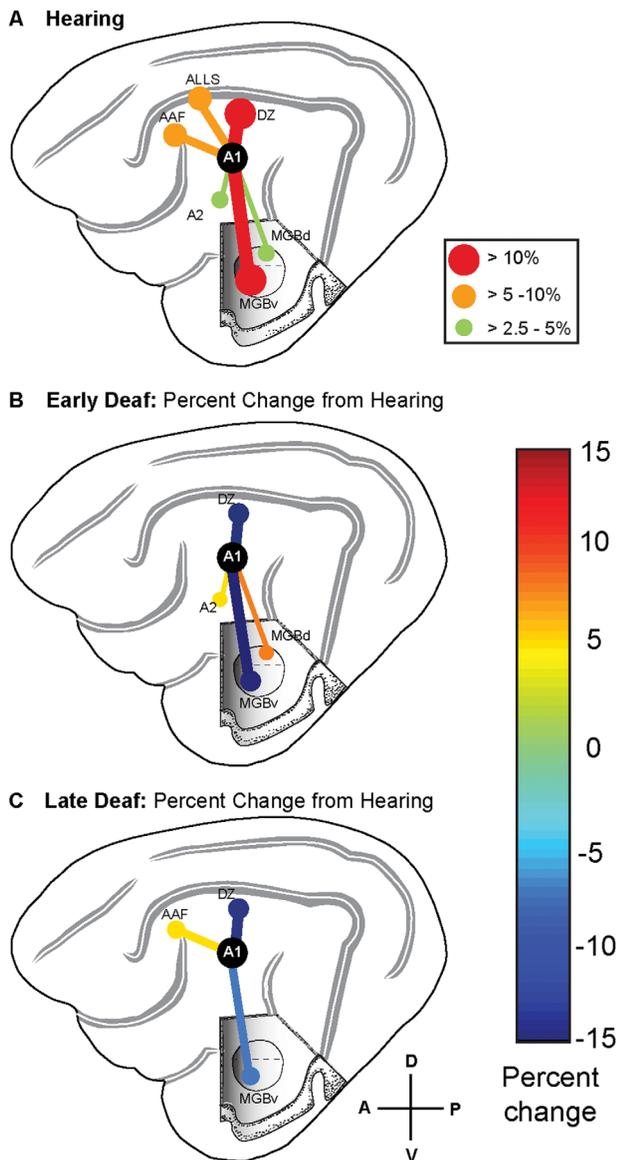


Figure 20. Corticocortical and thalamocortical projections to A1 that are affected by deafness. In the ventral region of the brain, the cortex has been “removed” to allow for visualization of the location of the medial geniculate body. **A:** In the hearing animals, the strength of the projections is represented by the size and color of the circles, with the largest circle representing an area that accounts for 10% or more of the total labeled projections to A1. **B,C:** In deaf animals, the changes in the strength of projections relative to control animals are represented by the size and color of the circles, with the largest circle representing an area that has undergone an increase or decrease in neuronal labeling of 10% or more, relative to controls. Note that statistically significant reorganization of projections to A1 occurs only in the auditory structures. For abbreviations, see list.

the medial nucleus (MGBm; hearing: 0.70%, early-deaf: 3.92%, late-deaf: 2.12%). Moreover, group differences were observed in both the ventral and dorsal nuclei. Following early-onset deafness, projections from MGBv

were significantly reduced relative to both control ($P < 0.001$) and late-deaf animals ($P < 0.001$), and those arising from MGBd were increased relative to control ($P = 0.002$) and late-deaf animals ($P = 0.042$). The proportion of projections from MGBv in the late-deaf animals was also decreased relative to control levels ($P = 0.014$). Thus, there were changes in the pattern of thalamic projections to A1, the nature of which was related to age at onset of deafness.

Although BDA-labeled neurons were identified in contralateral auditory cortical areas of each of the three groups examined, projections arose predominantly from the contralateral A1, and no group differences were present. Thus, contralateral data are not presented here.

DISCUSSION

Summary

This study demonstrates that overall patterns of projections to A1 are altered somewhat following deafness, and that the specific location and degree of reorganization is to some extent dependent on age at onset of deafness (Fig. 20b,c). Although a small-scale increase in projections from the somatomotor modality and decrease from the visual modality failed to reach statistical significance, within-modality changes were observed for some thalamic and cortical areas. Still, projections from many areas both within and across modalities appeared relatively unchanged following hearing loss. In the early-deaf, inputs from A2 were amplified, whereas the number of neurons originating in DZ was decreased. In addition, thalamic inputs from MGBd increased, whereas those from MGBv were reduced. In late-deaf cats, projections from AAF were amplified, whereas those from DZ decreased. In both early- and late-deaf, as well as in control animals, all interhemispheric connections arose from auditory areas, with more projections from A1 than any other area. Interestingly, whereas no similar labeling was observed in hearing animals, two early-deaf and two late-deaf animals showed projections to A1 originating in both area 17 of visual cortex and in the LP nucleus of visual thalamus, whereas one early-deaf animal showed projections from area 17 in isolation.

Although not significantly different at a group level, changes in the labeling in EPp were significantly correlated with an animal’s age at the onset of deafness. Overall, some anatomical evidence is provided for changes in the pattern of projections to A1 that are dependent on age at onset of deafness. Importantly, these changes include projections from nonauditory cortical areas, suggesting that an anatomical basis for

TABLE 1.
Primary Antibody Used

| Antibody | Immunogen | Source, Host Species, Cat#, RRID | Concentration |
|----------|-----------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|---------------|
| SMI-32 | Dephosphorylated epitope on medium- and high-molecular weight subunits of the neurofilament triplet | Sternberger Monoclonals, mouse monoclonal, Cat# SMI-32, RRID:AB_2315331 | 0.05 µg/ml |

functional crossmodal connectivity with primary auditory cortex exists.

Comparison with previous studies

The current study demonstrates that changes in the patterns of projections to A1 following deafness occur predominantly within the auditory modality. This includes both thalamic nuclei (MGBv, MGBd) and cortical auditory areas (A2, AAF, DZ), and is in accordance with expectations based on the proportion of projections arising from these areas in hearing animals (Paula-Barbosa et al., 1975; Imig and Reale, 1980; Morel and Imig, 1987; Rouiller et al., 1991; Scannell et al., 1995; He and Hashikawa, 1998; Lee and Winer, 2008a–c; Barone et al., 2013). Although Lee and Winer (2008a–c) have outlined thalamocortical and cortico-cortical connections to A1 in some detail, the current study is the first to examine how these projections change with acquired hearing loss across postnatal ages. Stanton and Harrison (2000) provided early evidence that thalamocortical projections to A1 were maintained following hearing loss; however, their animals had only slightly elevated thresholds at low frequencies, and they did not comment on corticocortical connections. Barone and colleagues (2013) compared A1 connectivity between congenitally deaf and normal hearing animals; however, their study included only two animals from each group; statistical models suggest that at such small sample sizes, it is impossible to distinguish group differences as they are obscured by between-animal differences (Scannell et al., 2000). Moreover, injections in the deaf animals of Barone and colleagues (2013) were seemingly not confined to A1 (one appeared to spread into DZ whereas the other was made along the A1/AAF border). The current study employed the necessary number of animals to make powerful between-group comparisons, and more closely controlled injection sites. Interestingly, these comparisons revealed differences that appear to depend on age at onset of deafness, which could only be observed by comparing patterns of change between early- and late-deaf animals.

Mechanisms of change

Decreases in the strength of intracortical connections, such as those originating in ipsilateral DZ follow-

ing both early- and late-onset deafness, are most easily understood as the result of Hebbian mechanisms through which neural connections that go unused are lost over time (Hebb, 1949). The cessation of stimulus-driven neural activity in the auditory system results in the absence of correlated neural activity at auditory synapses. This, in turn, leads to the weakening and potential pruning of those synapses. Similar decreases in functional connectivity have been reported in the visual cortex of blind subjects (Liu et al., 2007; Yu et al., 2008). Although the majority of inputs to DZ arise from thalamic nuclei, projections from A1 make up a significant proportion of the corticocortical afferents (He and Hashikawa, 1998; Lee and Winer, 2008c; Kok et al., 2014). Moreover, anatomical tracing suggests that information arising from A1 is integrated in DZ (Ojima and He, 1997). Thus, the susceptibility to pruning of projections from DZ to A1 fits well with data supporting a decrease in top-down modulation of auditory activity following deafness (see Kral and Sharma, 2012 for review) as well as data from the visual domain suggesting that corticocortical feedback pathways undergo long-term refinement (Batardiere et al., 2002), whereas feed-forward refinement is nearly complete early in prenatal life (Price et al., 2006).

More intriguing are those projections that are increased following deafness, including those arising from A2 and AAF. Anatomical evidence suggests that A2 is highly susceptible to reorganization, showing relatively large-scale increases in volume following both early- and late-onset deafness (Wong et al., 2013). The proportion of labeled cells in A2 was significantly larger in early-deaf animals than in either the control or late-deaf groups. A similar increase in the proportion of labeled neurons in A2 was also described following an injections of retrograde BDA in AAF (Wong et al., 2015). This may reflect residual exuberance in the early-deaf animals. In the developing brain, many more connections are formed than will persist into adulthood – a concept referred to as neuronal exuberance (see Innocenti and Price, 2005 for a review). Over time, networks are refined through the removal of ineffective or redundant connections in an experience-dependent manner. Thus, it is possible that the absence of acoustic experience from an early age prohibits the removal of superfluous connections, resulting in increased labeling relative to controls.

The early auditory experience of late-deaf animals appears to be sufficient to initiate the pruning process, resulting in a pattern of labeling that is similar to controls.

Alongside A1, AAF is a primary target of thalamocortical projections in the auditory system, with the two areas processing ascending acoustic information in parallel (Phillips and Irvine, 1982; see Hackett, 2011 for a review of processing hierarchies across species). In the current study, the proportion of labeled neurons in AAF increased following deafness. The small increase between the control and early-deaf groups failed to reach significance. However, the late-deaf group showed significantly more labeling than controls. One possible explanation for this increase in labeling is the formation of new projections between AAF and A1. However, this is extremely unlikely given the changes that occur in neuronal guidance mechanisms with age. In the embryonic nervous system, ephrins (molecules that direct the growth of neural projections) and their receptors are expressed at very high levels (Flanagan and Vanderhaeghen, 1998; Wilkinson, 2001). However, with the exception of areas that undergo routine remodeling throughout the lifespan (the olfactory bulb, hippocampus, and cerebellum; Liebl et al., 2003; Hafner et al., 2004), the expression of nearly all ephrins and ephrin receptors is dramatically decreased relative to developmental levels (Liebl et al., 2003). Thus it would seem unlikely that the type of concentration gradient required to guide new connections could be established in the adult brain.

It is far more likely that the increased labeling in AAF is the result of an increase in the number of synapses at existing junctions. An increase in synapses at A1 neurons would provide more opportunities for BDA uptake and, in turn, a higher probability of labeling in a given AAF neuron. Like neurogenesis, synaptogenesis is also most prevalent during sensitive periods for development (Rakic et al., 1986; Huttenlocher and Dabholkar, 1997); however, the formation of new synapses on existing neural structures occurs across the lifespan. Following the onset of ototoxic deafness, auditory-evoked neural activity is drastically reduced or eliminated. In response to the withdrawal of activity, the number of synapses between auditory structures may undergo compensatory increases to strengthen the response to any residual neural activity. This type of response might be expected in late-deaf animals, in which some period of hearing activity has solidified activity-dependent connections between cortical areas, and prevented substantial cross-modal plasticity in auditory structures. Such a response is made possible by the fact that, in contrast to pruning, synaptogenesis occurs largely in the absence of stimulus-driven activity (Wilson, 1988).

Whereas the majority of reorganization of cortical projections to A1 occurred intramodally, there was an interesting pattern of change in projections from the visual area EPp. Comparisons at the group level failed to reach significance (a power analysis using the data from the current study, a liberal measure of variance, and a Bonferroni corrected alpha value suggested that the group difference would reach significance with nine animals per group; Rosner, 2011). However, the proportion of labeling in EPp was significantly correlated to age at onset of deafness, with those acquiring hearing loss earliest showing a sharp increase in labeling, whereas those acquiring hearing loss later showed a pattern of labeling similar to control animals. EPp is part of the visual belt that lies posterior to auditory cortex and is thought to function as an auditory-visual associative area (Bowman and Olson, 1988), suggesting that projections from EPp to A1 are likely part of a feedback system. As was the case with feedback projections from A2, an increase in the early-deaf may reflect residual exuberant connections formed in early development. However, it should be noted that projections from other visual cortical areas (e.g., ALLS) appear to decrease following deafness, and that the magnitudes of these changes are large relative to those observed in EPp (yet still fail to reach statistical significance). Whatever the mechanism(s), these changes appear to be related to the age at deafness onset, and may impact the way in which A1 in animals with very limited auditory experience is activated by extramodal cortical areas.

Thalamic projections to A1

Across groups, the pattern of thalamic inputs was very similar; those from the medial geniculate body far exceeded any other thalamic nucleus. Moreover, within the MGB, the majority of projections to A1 originated in the ventral division, followed by the dorsal division, and finally, by the medial division. This is in accordance with anatomical data suggesting that the ventral division of the MGB projects mainly to A1 in both hearing cats and those with early-onset high frequency hearing loss (Stanton and Harrison, 2000), whereas primary auditory cortex is a secondary target of projections from the dorsal division (Winer et al., 1977), which projects primarily to A2 (Lee and Winer, 2008a).

There were differences in the pattern of projections from the subdivisions of the MGB between the early- and late-deaf groups. The proportions of labeled neurons in MGBv were reduced in both deaf groups relative to controls, with significantly less labeling in the early-deaf animals than in either other group. Conversely, there was an increase in labeling in the MGBd of early-

deaf animals relative to both the control and late-deaf groups. This difference between divisions of the MGB is not entirely surprising, given anatomical differences in the inputs to the two areas; whereas the MGBv receives afferent projections from the central nucleus of the inferior colliculus (Moore and Goldberg, 1963), projections to the dorsal subdivision originate in the lateral tegmental area (Casseday et al., 1976). Moreover, a recent study in which retrograde tracer injections were made into primary sensory areas (A1, V1, S1) suggests that age-related differences exist in the pattern of within- and between-modality connectivity between thalamic nuclei and sensory cortices (Henschke et al., 2014). There are also functional differences in response properties between the divisions (Calford, 1983), and in the targets of their projections. MGBv projects to A1, which is tonotopically organized, whereas the dorsal division projects to A2, which has no such organization. In early-deaf animals, there is a decrease in projections from MGBv and concurrent increases in projections from both MGBd and A2, suggesting that nontopographic connections are favored in deaf A1. This shift may be expected given that tonotopy in A1 likely serves little purpose in the absence of acoustic stimulation. Like the decreased labeling observed in projections from DZ, the decrease in the proportion of labeled cells in MGBv likely reflects pruning of projections that fail to stabilize in the absence of stimulus-driven activity. Contrary to feedback projections from DZ, which appear to be susceptible to removal following both early- and late-onset deafness, those originating in MGBv feed-forward to A1 and thus, are expected to undergo neural refinement early in life (Price et al., 2006). Indeed, the pattern of labeling observed in the current study would suggest that early deafness causes extreme reduction in these afferent projections, whereas deafness occurring later in life has a much smaller effect. Because the subdivisions of the MGB appear to represent separate functional pathways in the ascending auditory system (Winer et al., 1977), it follows that reorganization following deafness would be division-dependent.

In sum, when both thalamic and cortical projections to A1 are considered, changes in the pattern of BDA-labeled cells appear to occur primarily in projections from auditory structures. There is, however, an interesting pattern of change in projections for the visual-associative area EPP, which suggests that crossmodal influences on A1 may be dependent on age at onset of deafness. Additionally, projections from area 17 in visual cortex and the LP nucleus in visual thalamus were concurrently observed in a subset of early- and late-deaf animals, with no similar projections observed in hearing animals. Overall, the patterns of changes in thalamocortical and corticocortical

projections to A1 differed between early- and late-deaf animals, suggesting that changes in projections to A1 following deafness are age-dependent.

Reorganization of primary auditory cortex

The hearing cats in the current study show a pattern of projections from auditory cortical areas to A1 that is in accordance with what has been described previously (Paula-Barbosa et al., 1975; Imig and Reale, 1980; Rouiller et al., 1991; Scannell et al., 1995; He and Hashikawa, 1998; Lee and Winer, 2008b,c; Barone et al., 2013). The main auditory areas projecting to A1 are AAF, DZ, A2, and PAF, whereas those areas with the fewest labeled cells include areas IN, T, and FAES. Importantly, the strongest projections to A1 originate in areas that immediately surround A1, whereas more distant areas form weaker projections. A similar proximity-based pattern of projections to A1 was also described by Imig and Reale (1980).

Sources of somatomotor and visual inputs to the auditory cortex have not been studied extensively in the hearing cat. Clemo and colleagues (2008) described a projection from A1 to the posterolateral lateral supra-sylvian area (PLLS), a visual structure; the current study provides evidence for a reciprocal connection in the hearing cat. Functional evidence for auditory cortical activity evoked by somatomotor and visual stimulation has been consistently demonstrated in monkeys (Schroeder et al., 2001; Foxe et al., 2002; Schroeder and Foxe, 2002, 2005; Fu et al., 2003; Kayser et al., 2005), whereas anatomical evidence exists for connectivity between nonauditory modalities and auditory cortical areas in both the monkey (Cappe and Barone, 2005; Hackett et al., 2007) and Mongolian gerbil (Budinger et al., 2006, 2008; Budinger and Scheich, 2009). However, no study has examined these cross-modal inputs to A1 in the hearing cat. The somatomotor areas projecting to A1 in the current study are similar to those described in these aforementioned species. The strength of somatomotor projections to A1 did not differ between hearing and deaf animals.

These data show an intramodal connectional reorganization following the loss of auditory input. Overall, the normal pattern of connections is maintained in the absence of auditory input; some areas, like A2 and AAF, undergo increases in the strength of connections, whereas others, such as DZ, are decreased in strength following deafness. Intramodal plasticity following deafness is not surprising given recent anatomical evidence of volumetric changes in deaf cats. For example, many auditory areas have been shown to undergo boundary shifts, leading to changes of areal volume (Wong et al., 2013). For instance, in the early-deaf cat, A2 occupies a

bigger territory in the auditory cortex due to a dorsal shift of its dorsal boundary with A1. This increase in the size of A2 in the deaf may be linked to the increase in the strength of the projection from A2 to A1 reported in this study. Interestingly, intramodal reorganization following the loss of sensory input is not unique to the auditory cortex. The primary somatomotor cortex of the monkey can be reactivated by nonprimary somatomotor afferents following a complete lesion of the dorsal column of the spinal cord (Qi et al., 2011; Boves et al., 2012, 2013).

In sum, the preponderance of the available evidence from electrophysiology (Allman et al., 2009; Meredith et al., 2012), functional imaging (Finney et al., 2001; Auer et al., 2007; Scott et al., 2014), and neuroanatomical studies (Wong et al., 2015; present study) suggests that A1 undergoes reorganization following deafness. Although two studies have failed to observe crossmodal plasticity in A1 of the deaf cat, it is possible that the small sample size tested ($n = 1$; Stewart and Starr, 1970) or the type of anesthetic used (halothane; Kral et al., 2003) could be responsible for the lack of responsiveness of A1 rather than a lack of crossmodal plasticity per se, as the current study suggests that some intermodal connections are altered following deafness.

Ectopic or not ectopic: that is the question

In the current experiment, a subpopulation of deaf animals shows the presence of a connection from area 17 to A1 that is not present in hearing controls. However, a connection between V1 and A1 in hearing animals cannot be excluded based on the current study. Previous work has demonstrated that reciprocal projections (those from A1 to area 17) are present in hearing cats (Hall and Lomber, 2008), although there is an argument to be made that projections from A1 to V1 may be more functionally relevant than the reverse based on the relative arrival time of information in cortex. Regardless, an electrophysiological study by Schroeder and Foxe (2002) describes visual activation of auditory cortex in three of five hearing monkeys. The discrepancy between this work and the current study may be related to the location and extent of the tracer spread in the current study. The ventral border of A1 has been shown to shift dorsally in deaf cats (Wong et al., 2013). Thus, during injections, the pipette was placed along a plane lying dorsal to the tips of the posterior and the anterior ectosylvian sulci to ensure that injections were made into A1 in all groups. However, with the volume of tracer per injection held constant, and with similar spread, injections in the current study did not permeate A1 to an equal extent in every group. In hearing cats, only the central portion of A1 was filled, whereas the deaf cats received tracer injections

encompassing both central A1 and the ventral portion that abuts A2. In addition, the cats that showed the ectopic connection with area 17 in the current study also showed more posterior spread of the tracer than cats that did not show this connection. Interestingly, the boundary of A1 and the secondary auditory cortex at the posterior end is the same area described by Schroeder and Foxe (2002) as being involved in visual activation of the unisensory auditory cortex in monkeys.

Connections between area 17 and the auditory cortex were first described by Innocenti and Clarke (1984) as transient in nature. Since then, however, growing evidence has suggested that these connections may stabilize over time. Animal models of neural rewiring provide elegant evidence that crossmodal connections are fully functional (Frost, 1982; Sur et al., 1988; Pallas et al., 1990). Importantly, anatomical evidence of crossmodal connections has been observed across a number of intact animal species including monkeys (Cappe et al., 2009), mice (Charbonneau et al., 2012), ferrets (Bizley et al. 2007), and gerbils (Budinger et al., 2006; 2008; Budinger and Scheich, 2009). In addition, electrophysiological evidence suggests that connections between the visual and auditory cortices are functional in normal hearing ferrets (Bizley and King, 2008) and monkeys (Schroeder and Foxe, 2002). Another pathway that may contribute to the visual activation of A1 is the subcortical connection observed from LP to A1 in some deaf animals. The LP nucleus is a visual thalamic relay. That very same connection was recently shown to exist in the congenitally deaf white cat by Barone et al. (2013), who have suggested that projections between LP and V1 may contribute to the transmission of nonauditory information to the auditory cortex of the deaf cat. A change in the subcortical connectivity is not uncommon following the loss of a sensory modality. For example, the visual cortex of the enucleated opossum receives input from nuclei associated with the somatomotor (ventral posterior nucleus, VP) and auditory (MGB) systems (Karlen et al., 2006).

CONCLUSIONS

Altogether, the results of the current study support the idea that whereas projections from many cortical areas to A1 appear unchanged, some thalamocortical and corticocortical projections are reorganized following deafness. The age at onset of deafness has a pronounced effect on the pattern of projections that results from such reorganization. The data presented here are essential for improving our understanding of the anatomical mechanisms that underlie adaptation of the auditory cortex to deafness. Critically, anatomical evidence is

provided for crossmodal connectivity involving primary auditory cortex, and for small-scale changes in these connections following deafness that might underlie functional crossmodal activity in deaf A1. More studies are needed to elucidate the type of information that is being conveyed in these altered connections to A1. Moreover, converging lines of evidence suggest that changes in projections targeting and originating in A2 may play an important role in adaptive reorganization.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ROLE OF AUTHORS

All authors had full access to all of the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: NC and SGL. Acquisition of data: BEB, NC, and SGL. Analysis and interpretation of data: BEB and NC. Drafting of the manuscript: BEB and NC. Critical revision of the manuscript for important intellectual content: BEB and SGL. Statistical analysis: BEB and NC. Obtained funding: SGL. Study supervision: SGL.

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