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Is territorial expansion a mechanism for crossmodal plasticity?

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Abstract

Crossmodal plasticity is the phenomenon whereby, following sensory damage or deprivation, the lost sensory function of a brain region is replaced by one of the remaining senses. One of several proposed mechanisms for this phenomenon involves the expansion of a more active brain region at the expense of another whose sensory inputs have been damaged or lost. This territorial expansion hypothesis was examined in the present study. The cat ectosylvian visual area (AEV) borders the auditory field of the anterior ectosylvian sulcus (FAES), which becomes visually reorganized in the early-deaf. If this crossmodal effect in the FAES is due to the expansion of the adjoining AEV into the territory of the FAES after hearing loss, then the reorganized FAES should exhibit connectional features characteristic of the AEV. However, tracer injections revealed significantly different patterns of cortical connectivity between the AEV and the early-deaf FAES, and substantial cytoarchitectonic and behavioral distinctions occur as well. Therefore, the crossmodal reorganization of the FAES cannot be mechanistically attributed to the expansion of the adjoining cortical territory of the AEV and an overwhelming number of recent studies now support unmasking of existing connections as the operative mechanism underlying crossmodal plasticity.

Graphical abstract

Following sensory loss (e.g., deafness), a proposed mechanism underlying crossmodal plasticity is that the territory of a deprived brain region (area “A”) is taken over by neighboring, more active regions of the brain (enlarged area “B” - bottom left). However, the present study determined that a crossmodally reorganized (“Reorganized area A”) cortical region retains its areal distinctions (connectivity, cytoarchitectonics and function - bottom right), which is not consistent with being incorporated (large X) into another area (area “B”).

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Keywords

Hearing loss; Visual cortex; Auditory cortex; Sensory substitution; Cortical connectivity

INTRODUCTION

It is well known that individuals who lose function in one sensory modality (e.g., blindness, deafness) often show compensatory improvements in the performance of the remaining, intact sensory systems. Not only has such crossmodal plasticity been identified in humans (Bavelier and Neville, 2002; Merabet & Pascual-Leone, 2010; Frasnelli et al., 2011), but in experimental animals as well (Rauschecker, 1995; Lomber et al., 2010, 2011; Meredith et al., 2011; Butler and Lomber, 2013). However, the neural mechanisms underlying crossmodal plasticity are poorly understood and, until recently, have rarely been examined. One of several hypothetical mechanisms proposed to subserve crossmodal plasticity is that of territorial expansion as summarized in Figure 1. Stated simply, if an area “A” loses its particular sensory activation, an adjacent active sensory area (e.g., area “B”) expands to occupy the vacated area “A.” Accordingly, in the seminal study of experimental crossmodal plasticity, it was proposed that “visual deprivation led to an expansion of the neighboring nonvisual areas into normally visual territory (Rauschecker, 1995).” Several factors are critical to this hypothesis. One is the definition of “area” itself. A cortical “area” or territory is functionally distinct, has a unique topographic map, exhibits particular receptive field properties, receives a specific set of connections, and exhibits a characteristic cytoarchitectonic or histochemical pattern (Rosenquist, 1985). In addition, the deprived area must share a border with a representation of another sensory modality across which the expansion occurs. In the context of crossmodal plasticity, for sensory loss to induce a hypothetical region “B” to expand and overtake the ‘abandoned’ territory of area “A,” the territory of “A” must not only change functionally, but it must also modify its connectional and cytoarchitectonic features to resemble those of area “B.” If not, despite their functional similarities, the crossmodally reorganized region cannot be regarded as an expansion of the other. To our knowledge, however, such an empirical examination of the territorial expansion hypothesis of crossmodal plasticity has not been conducted.

Within the cerebral cortex, a great deal is known regarding the different sensory areas represented in the cat anterior ectosylvian sulcal (AES) region. The ectosylvian visual area

(AEV; Mucke et al., 1982; Olson and Graybiel, 1987; Wallace et al., 2006) is located along the postero-ventral bank of the sulcus while the auditory field of the anterior ectosylvian sulcus (FAES; Clarey and Irvine, 1990a; Meredith & Clemo, 1989; Wallace et al., 2006) is found in the postero-dorsal bank. Within the AEV, neurons are preferentially responsive to small ($\sim 1^\circ$ diameter), rapidly moving spots of light (Mucke et al., 1982) or gratings and tend to show directionally selective activity to visual stimuli moving toward the midline (Scannell et al., 1996). Visual receptive fields in AEV tend to be large ($>40^\circ$ diameter) but collectively do not exhibit an obvious retinotopy (Mucke et al. 1982, Olson and Graybiel 1983; Scannell et al. 1996; Wallace et al., 2006). Cortical inputs to the AEV are derived from several ipsilateral visual cortical areas (Mucke et al. 1982; Reinoso-Suárez and Roda 1985; Norita et al. 1986; Olson and Graybiel 1987; Grant and Shipp 1991) but predominantly arise from the posterolateral lateral suprasylvian area (Olson and Graybiel, 1987). In addition, the AEV has been cytoarchitectonically distinguished from its neighboring regions (van der Gucht et al. 2001) such as the anterior auditory field (AAF) and the FAES (Mellott et al., 2010; Wong et al., 2014). The AEV shares a common border with the auditory FAES, whose neurons largely respond to acoustic stimulation (Clarey and Irvine, 1990a; Meredith and Clemo, 1989) as depicted in Figure 2 (top) and are also sensitive to sound location (Clarey and Irvine, 1990a; Korte and Rauschecker, 1993; Xu et al., 1998; Carriere et al., 2007; Las et al., 2008) or sound motion (Jiang et al., 2000). Functionally, the FAES is the major source of auditory corticotectal projections (Meredith and Clemo, 1989; Chabot et al., 2013; Butler et al., 2016a) which is consistent with its role in SC-mediated auditory orienting behaviors (Malhotra and Lomber, 2004; Meredith et al., 2011). The FAES receives inputs largely from other ipsilateral auditory cortices, especially the AAF and the secondary auditory area (A2; Clarey and Irvine, 1990b; Lee and Winer, 2008; Meredith et al., 2016). The FAES has been cytoarchitectonically distinguished from the regions bordering it, such as the anterior auditory field and the AEV (Mellott et al., 2010; Wong et al., 2014). Thus, in normal, hearing cats the adjoining AEV and FAES regions are functionally, connectionally and cytoarchitectonically distinct from one another.

Functional distinctions between the AEV and FAES, however, seem to disappear for visually-deprived cats (Rauschecker and Korte, 1993) or for animals deafened prior to the onset of the auditory critical period of development (Meredith et al., 2011). As illustrated in Figure 2, auditory-evoked activity in the FAES is replaced in early-deaf animals largely ($>70\%$) by visual response activity. In this crossmodally-reorganized FAES of early-deaf cats, visual receptive fields are large (average 56° diameter) and display complex response properties such as direction and velocity preferences. In addition, the visual receptive fields strongly represent the central visual field and collectively encompass the contralateral visual field, although a retinotopic organization was not observed (Meredith et al., 2011). Thus, the features of visually-reorganized FAES are strikingly similar to visual features of the adjoining AEV. Therefore, it seems possible that the visual crossmodal plasticity observed in the FAES following early hearing loss may occur as an expansion of the nearby visual representation in the AEV, as proposed for the effects following early visual deprivation (Rauschecker and Korte, 1993). If these functional changes in the early-deaf FAES are the result of territorial expansion of the AEV, then the visually-reorganized FAES in the deaf should exhibit the connectivity and cytoarchitectonic pattern of the AEV. This possibility is

examined in the present study using neuroanatomical tracer injections to compare the features of the visual AEV area with those of the visually-reorganized territory of the early-deaf FAES.

MATERIALS AND METHODS

All procedures were performed in compliance with the *Guide for Care and Use of Laboratory Animals* (National Institutes of Health, publication 86-23), the National Research Council's *Guidelines for Care and Use of Mammals in Neuroscience and Behavioral Research* (2003) with prior approval by the Institutional Animal Care and Use Committee at Virginia Commonwealth University. Also, all procedures were conducted in accord with the Canadian Council on Animal Care's *Guide to the Care and Use of Experimental Animals* (Olfert et al., 1993) and were approved by the University of Western Ontario Animal Use Subcommittee of the University Council on Animal Care.

Ototoxic procedures

All early-deaf animals were obtained from pregnant mongrel cats to avoid potential genetic influences on neural connectivity that may be coupled with congenitally deaf lineages. At 6–8 days postnatal (near hearing onset for cats), each animal was deafened using the ototoxic protocol of Xu et al. (1993). Inhalation anesthesia (isoflurane) was used to permit catheterization of the saphenous or jugular vein. A single, subcutaneous dose of kanamycin (300 mg/kg) was then administered followed by the intravenous injection of ethacrinic acid (100mg/kg). Following recovery, the animals were returned to their mother as quickly as possible where they were housed until they were weaned (~6 weeks postnatal).

Hearing evaluation

At 4–6 weeks postnatal, treated animals had their hearing tested using standard auditory brainstem responses (ABR). Under ketamine (30mg/kg) and acepromazine (5 mg/kg) anesthesia, a calibrated auditory click (at least 2000 trials each, 0.1 ms square-wave click, rarefaction) delivered through a minispeaker positioned in front of the ear was used as the auditory stimulus. The full range of stimulation intensities was run for one ear before presenting the tests to the other ear. Subdermal recording leads were placed at sites superior to the mastoid processes of the right and left ears, at a mid-cranial scalp location, and at a mid-back position. Electrical activity recorded by the leads was routed through an amplifier to a computer for signal averaging and storage. Animals with an ABR threshold of >90 dB SPL in both ears, as depicted in Figure 3, were defined as profoundly deaf in accordance with the criteria of the World Health Organization (1991). Deafened animals were raised into the period of auditory maturity (>6 months of age; Kral et al., 2005).

Neuroanatomical tract-tracing procedures

Tracer injections were conducted on adult cats (>6 months of age; Kral et al., 2005). In three cats (weight range 2.9–3.4kg) with no developmental sensory restriction, the visual area AEV was injected. In three (weight range 3–3.5kg) early-deaf cats, the visually-reorganized area FAES was injected. The injection sites of each of the cases included in this study are illustrated in Figure 4. For injection, the animals were anesthetized (sodium pentobarbital,

30 mg/kg i.v.) and their heads were secured in a stereotaxic frame. Under aseptic conditions, a unilateral craniotomy was made to expose the AES cortex. An electrode carrier was used to support a syringe (Hamilton 5 μ l; 31-gauge needle) containing the tracer biotinylated dextran amine (BDA; lysine fixable; 10% in PBS). The carrier was angled 53–60° (from vertical) with 35–40° cant (anterior-to-posterior from the coronal plane) and the needle tip was inserted at a point 0.8 to 1.5 mm anterior to the vertical limb of the AES to a depth of 5–7 mm. The tracer was ejected to a total volume of 0.7–1.3 μ l. After the injection was complete and the needle was retracted, the exposed cortical surface was packed with gelfoam, the incision was sutured closed, and standard postoperative care (analgesia, thermal and fluid support) was provided. As designated by the case numbers indicated in Figure 2 (bottom) and Figure 4, tracer injections into FAES were conducted in two of the same early-deaf animals whose FAES crossmodal reorganization had been electrophysiologically demonstrated (reported in Meredith et al., 2011).

Histological processing

After a 7–10 day post-injection period for tracer transport, the animals were deeply anesthetized (sodium pentobarbital, 40 mg/kg, i.v.) and perfused transcardially with heparinized saline followed by fixative (4.0% phosphate buffered paraformaldehyde). The brain was exposed, blocked stereotaxically, removed and cryoprotected (25% sucrose in 0.1M phosphate buffer). Coronal sections (50 μ m thick) were cut using a freezing microtome and collected serially from the coronal sulcus of the cortex. A series of sections (at 250–300 μ m interval) was processed for visualization of BDA after the protocol of Veenman et al. (1992) with heavy metal intensification. Reacted sections were mounted on treated slides, dehydrated and coverslipped without counterstain. An additional series of sections was counterstained using cresyl violet to assist in cytoarchitectonic and laminar identification. In each of the cases labeled tracer was observed as distant as the midbrain (which is known to connect with the injected areas) and our lab has used these methods successfully in other published studies of cortical connectivity (Meredith and Clemo, 1989; Clemo et al., 2008; Allman et al., 2009; Meredith and Allman, 2012; Meredith et al., 2016).

Data Analysis

Neuronal labeling was visualized using a light microscope (Nikon Eclipse-600) that was also equipped with a PC-driven digitizing stage controlled by NeuroLucida software (MBF Biosciences, Williston VT) for plotting the data. Using this device, a calibrated tracing was made of each tissue section that included its outline, the border between gray and white matter, the position and extent of the injection site, and retrogradely labeled neurons. The injection site was defined as the large aggregate of densely labeled cell bodies and neuropil at the end of the injection needle track. BDA-labeled neurons were sharply black throughout their soma and, sometimes, distal dendrites and only intact, labeled cell bodies were counted. Labeled neurons were plotted at 200X magnification and NeuroLucida kept a count of the numbers of neurons marked. In this manner, an entire series of sections were plotted at regular intervals (500–600 μ m) through cortex for each case. Data plots were converted to a graphic format and exported to a graphics program for visual display and comparison.

A cat stereotaxic atlas (Reinoso-Suárez, 1961) that was updated with information from more recent studies and reviews (Avendano et al., 1988; Bowman and Olson, 1988; Clasca et al., 1997; Clemo and Meredith, 2004; Lee and Winer, 2008; Lomber and Malhotra, 2008; Mellott et al., 2010; Meredith, 2004; Mucke et al., 1982; Payne, 1993; Ribaupierre, 1997; Rosenquist, 1985; Updyke, 1986; van der Gucht et al., 2001) was used as a template to define the functional subdivisions of cortex based on AP-position and gyral/sulcal patterns. A list of abbreviations for the names of the cortical functional subdivisions is provided in Table 1. These functional divisions were used to tabulate counts of retrogradely labeled neurons by region and the total number of counted neurons was normalized to represent 100% of the ipsilateral cortical projection by case. These regional values were then grouped by treatment (AEV injection site vs. early-deaf FAES injection site) and statistically assessed (mean per cortical region $\pm$ standard error) and compared (Wilcoxon test) using JMP12 statistical software (SAS Institute, Cary NC). Tissue labeled by FAES tracer injection was re-examined from animals archived from archived tissue from an earlier study. In addition, neurons that project from AEV to FAES were measured for soma size using NeuroLucida by tracing the cell body perimeter (at 400x) and using the NeuroExplorer program to calculate the cell body area (μm^2). Every labeled neuronal cell body within the defined area was measured from hearing (n=3) and from early-deaf (n=4) cats from tissue used for a published study of FAES connectivity (Meredith et al., 2016). Data was then tabulated and statistically compared using a Wilcoxon test.

RESULTS

Injection Sites

It has been noted in other studies of the AES cortex that the sulcus itself exhibits substantial inter-animal variability (Clemon and Stein, 1983; Meredith et al., 2016), rendering fixed stereotaxic approaches ineffective. Hence, examination of these cortices is dependent on post-experimental confirmation of the injection site. Accordingly, although numerous experimental attempts were made, tracer injections in 6 cats were confirmed of being either within the AEV (n=3) or in the early-deaf FAES (n=3). As shown in Figure 4, each of the AEV injection sites occupied essentially the full-thickness portion of the ventral sulcal wall that corresponds with electrophysiological localization of the AEV region (Mucke et al., 1982; Norita et al., 1986; Olson and Graybiel, 1987). Each of the tracer injections into the territory of the FAES successfully filled the dorso-medial sulcal wall that corresponds with the electrophysiologically-determined location of the FAES (Meredith and Clemon, 1989; Las et al., 2008; Meredith et al., 2006, 2011; Meredith and Allman, 2009). Tracer injections into AEV or early-deaf FAES retrogradely labeled neurons, most often with pyramidal morphology, in regions of ipsilateral cortex, as demonstrated in Figure 5.

Cortical Projections to AEV

In accordance with previous examinations of AEV (Mucke et al., 1982; Norita et al., 1986; Olson and Graybiel, 1987), sources of cortical inputs to AEV originate largely within other visual areas, in particular those in the lateral suprasylvian visual cortical region. Figure 6 (top) illustrates one such case, where neurons retrogradely labeled from AEV are prevalent within the visual regions of the ALLS, PMLS and PLLS regions. The data from all 3 cases

with tracer injected into area AEV is summarized in Figure 7 (grey bars), which quantifies the preponderance of inputs arising from areas ALLS, PMLS and PLLS, but also includes proportionally smaller contributions from many other visual cortical regions, including VLS, PS and lower-level cortical areas 17, 18 and 19. Labeled neurons found within AEV are not included because these represent intrinsic connections and also could result from direct labeling due to proximity to the injection site. In contrast to its visual cortical connections, Figure 6 (grey bars) indicates that auditory cortical regions A1, A2, DZ, PAF, and TE are sparsely, if at all, connected with AEV.

Cortical Projections to Early-deaf FAES

Sources of ipsilateral cortical inputs to the FAES in an early-deaf animal are depicted in Figure 6 (bottom), which included the visual areas ALLS and PLLS. These visual cortical connections are quantitatively summarized for all 3 early-deaf FAES cases in Figure 5 (black bars), which shows that inputs from visual areas consistently arise from the nearby AEV with proportionally few inputs arising from ALLS and PLLS, and virtually none from the other visual cortical regions. Furthermore, despite losing its auditory function due to deafness, the early-deaf FAES maintains robust connections with numerous auditory cortical areas, including AAF, A1, A2, and DZ, as shown in Figure 6 (bottom) and quantitatively summarized in Figure 8 (black bars). The connectional patterns of the FAES in hearing cats (documented in previous studies: Clarey and Irvine, 1990; Lee and Winer, 2008; Meredith et al., 2016) primarily include other auditory areas such as AAF and A2, which is a pattern like that of the early-deaf FAES.

It is possible that collateral branches from AEV to deafened FAES might underlie a post-deafness expansion of the AEV. If enhanced axonal branching in the AEV-to-FAES projection occurs, this phenomenon should correlate with increase in soma size (to accommodate and support the added synaptic structures) of AEV neurons that project to the deafened FAES. To test this idea, the cell body size of AEV neurons projecting to FAES was measured and compared between hearing and early-deaf animals. The average soma size for hearing (mean = $155.4 \pm 2 \mu\text{m}^2$ se; $n=839$) and for early-deaf (mean = $152.7 \pm 2.5 \mu\text{m}^2$ se; $n=547$) was not statistically different (Wilcoxon, $p=0.46$), suggesting that exuberant branching of AEV axon collaterals was not likely to subserve the crossmodal reorganization of FAES.

Comparison of Cortical Projections to AEV and Early-deaf FAES

Because the FAES becomes visually reorganized in the early-deaf animals, it might be expected that the visually reorganized FAES territory might acquire the same visual inputs as its visual neighbor, the AEV. However, as depicted in Figure 7, the proportions of visual cortical projections to these areas are significantly different for nearly all regions of origin. Specifically, the projection from PLLS is the most prevalent visual cortical input to AEV, representing 20% of the total ipsilateral cortical projection, but this same area represents only 3% of the inputs to the early-deaf FAES, which is a statistically significant (Wilcoxon, $p=0.04$) difference. Similarly, the projection from PMLS to AEV occupies 8% of the inputs to AEV, yet none were observed into the early-deaf FAES (Wilcoxon, $p=0.04$). This pattern of smaller, significantly smaller or completely absent visual projections into early-deaf

FAES occurred for each visual area examined, despite its visual crossmodal reorganization. On the other hand, as demonstrated in Figure 8 relatively few ipsilateral auditory cortices connect with AEV, although the early-deaf FAES reveals plentiful inputs from most ipsilateral auditory cortical regions. When auditory cortical connections are compared for AEV and early-deaf FAES, significantly (Wilcoxon, $p < 0.05$) lower connectivity is observed for inputs to AEV from auditory areas A1, A2, and DZ. Specifically, projections from auditory areas AAF, A1 and DZ to early-deaf represent 9.5%, 6.9% and 5.9% of the total projections to the region, but only 1.35%, 1.34% and 0.3%, respectively, to AEV. Thus, a general pattern of significantly larger auditory projections occurs for early-deaf FAES than are observed for the AEV. In general, overall projections from visual cortical areas to AEV represent >51% of its total cortical connections, but visual cortical projections to early-deaf FAES constitute only 19% of the total cortical inputs. Ultimately, when these data are comprehensively compared in Figure 9, the radial plots that include data from each examined cortical region (whether a connection was observed or not) demonstrate that the pattern of cortical connectivity is fundamentally different for the AEV and the early-deaf FAES.

DISCUSSION

Following sensory deprivation such as deafness, crossmodal reorganization is a widespread phenomenon across core (e.g., Finney et al., 2001; Hunt et al., 2006; Auer et al., 2009; Allman et al., 2009; Meredith and Lomber, 2011; Meredith and Allman, 2012; Ouda et al., 2016) as well as higher-order cortices (Lomber et al., 2010; Meredith et al., 2011; Land et al., 2016) and subcortical regions (Dehmel et al., 2008; Shore et al., 2008). Furthermore, hearing loss-induced crossmodal plasticity is a robust phenomenon that occurs in cases of congenital (Lomber et al., 2010; 2011; Land et al., 2016), post-natal (Meredith and Lomber, 2011; Meredith et al., 2011) and adult (Allman et al., 2009; Meredith et al., 2012) deprivation and is sensitive to even modest levels of sensory loss (Shore et al., 2008; Meredith and Allman, 2012). Until recently, the neuronal mechanisms underlying crossmodal plasticity have received more speculation than empirical examination. Some of notions proposed to underlie crossmodal plasticity include the enhanced ingrowth of projections from novel sources, or the unmasking or reweighting of existing inputs that were otherwise silent (e.g., Rauschecker, 1995; Bavelier and Neville, 2002). Several recent studies of deafness-induced crossmodal plasticity failed to find significant support for the “novel projections” hypothesis (Meredith and Allman, 2012; Kok et al., 2013; Barone et al., 2013; Wong et al., 2015; Chabot et al., 2015; Meredith et al., 2016). Instead, these same studies indicate that connections with auditory cortical sites in early-deaf animals largely resemble those of their hearing counterparts, suggesting a role for the re-weighting of existing inputs (Meredith et al., 2016). However, none of these studies were designed to address the possibility that crossmodal plasticity might result from the “expansion of the more active... brain region at the expense of another...region” (Rauschecker, 1995). The present study examined the cortical sources of inputs to visual AEV and that of its deafened neighbor, the auditory FAES. As documented in Figure 9, these experiments demonstrated that AEV receives substantially different cortical inputs than the early-deaf FAES. Therefore, despite their proximity and similarly visual functions, their significant differences in connectivity

indicate that the early-deaf FAES should not be regarded as a territorial expansion of the AEV.

Further support for this conclusion is apparent in the cytoarchitectonic distinctions and regional borders established for these two regions. The cytoarchitectural features of the visual AEV have been described as “layer V contains occasional darkly stained neurons for SMI-32, whereas layer III mainly consists of smaller pyramidal neurons that are more densely packed in the deeper half of this layer.... Furthermore, layer VI is specified by a weak neuropil staining and a fairly low density of labeled fibers...” as depicted in Figure 12 of van der Gucht et al., (2001). The cytoarchitectonic properties of the FAES have been described as exhibiting “a wide band of [SMI-32] immunoreactivity...due to the widening of supragranular layers...and high labeling density of FAES layer III across all experimental groups” as presented in Figure 4B of Wong et al., (2014; also depicted in Figure 12A of van der Gucht et al., 2001; Figure 13 in Mellott et al., 2010). Despite their focus on modality-specific representations (e.g., visual only, auditory only) both van der Gucht et al. (2001) and Mellott et al., (2010) plotted a distinct border between AEV and FAES in hearing cats. In addition, in a study of cartographic changes that follow early-deafness, a cytoarchitectonic change between AEV and the territory of the early-deaf FAES also plots across the fundic region of the AES (see Figure 11A, Wong et al., 2014). However, the location of the border between AEV and the territory of the early-deaf FAES is similar, but not identical to that identified in hearing animals (Figure 11A, Wong et al., 2014), and this observation has been interpreted as indicative of changes in cartographic distribution of the FAES in the early-deaf. Ultimately, the identification of the border between these regions in early-deaf animals is based upon the cytoarchitectonic differences between the two areas, which does not support the notion that the early-deaf FAES is overtaken by an expanded AEV.

Behavioral observations also do not support the territorial expansion hypothesis as a mechanism of crossmodal plasticity in these regions. Cats trained to detect and localize visual stimuli do not exhibit visual localization deficits when the AEV is functionally deactivated (Lomber and Payne, 2004; Malhotra and Lomber, 2004; Lomber and Malhotra, 2008). In contrast, functional deactivation of the FAES in hearing animals blocks localization behaviors evoked by auditory stimuli but not visual stimuli (Malhotra and Lomber, 2004; 2007; Lomber and Malhotra, 2008; Meredith et al., 2011). In addition, deactivation of the territory of FAES in early-deaf animals eliminates localization behaviors elicited by visual stimuli (Meredith et al., 2011). Given these substantial differences in behavioral involvement, the visual reorganization of FAES following early-deafness (which is critical for localization behaviors) cannot be regarded as an expansion of the AEV (which has no demonstrable role in localization).

Ultimately, comparisons of published corticocortical connectional studies with features similar to the present investigation also do not lend support to the territorial expansion hypothesis of crossmodal plasticity. Although the Dorsal Zone of auditory cortex (area DZ; Stecker et al., 2005) shares a border with the posterolateral suprasylvian visual area (PLLS), visually reorganized early-deaf DZ (Lomber et al., 2010; Land et al., 2016) does not exhibit the same connectional pattern observed for PLLS. Specifically, the visual PLLS receives heavy projections from visual areas AEV, DLS and PS and lighter levels of input from visual

areas 17, 18 and 20a (Symonds and Rosenquist, 1984; Scannel et al., 1995). In contrast, visually-reorganized early-deaf DZ receives only a small input (2%) from DLS and all the other visual cortical regions (AEV, PS, 17, 18, 20a) provide weak (<1%) or no input (Kok et al., 2013; see also Barone et al., 2013). Likewise, auditory area Anterior Auditory Field (AAF) has been demonstrated to exhibit somatosensory and, to a lesser extent, visual crossmodal reorganization following deafness (Meredith and Lomber, 2011). The AAF shares a border, anteriorly, with somatosensory area S2 and medially, with Anterolateral Lateral Suprasylvian visual area (ALLS). Following deafness, the reorganized AAF receives approximately 14% of inputs from somatosensory area S2, 6% from somatosensory S4 (with few to no inputs from areas S1, S3 or S5; Wong et al., 2015). In contrast in hearing animals S2 receives strong inputs from S1 and motor cortex, with none from areas S3 or S5 (Burton and Kopf, 1984). A similar pattern occurs for visual connectivity to AAF, where about 6% of inputs to crossmodally-reorganized early-deaf AAF arrive from visual ALLS and 2.6% from visual AEV (with virtually none from visual areas AMLS, PMLS, PLLS, 17, 18, or 19; Wong et al., 2015) while ALLS in hearing animals receives strong inputs from PLLS and AEV with minor inputs from areas AMLS, 19 and 18 (Symonds and Rosenquist, 1984). Hence, neither the somatosensory nor visual connectivity of reorganized AAF resembles that of its neighboring somatosensory (S2) or visual (ALLS) regions. Therefore, like the present study, these additional comparisons fail to support the territorial expansion hypothesis of crossmodal plasticity.

Although not the same as territorial expansion, another possible mechanism to account for the functional similarity of AEV and the visually reorganized early-deaf FAES might be through increased sprouting of local collaterals from the AEV to the FAES. The present study observed that projections from AEV to early-deaf FAES represents an average of 6.4% ($\pm 2.7\%$ sd) of the total ipsilateral cortical projection to the region, while 9.5% ($\pm 0.8\%$ sd) arise from AEV in hearing animals (Meredith et al., 2016). This small reduction in projection size is inconsistent with the notion that an increase in AEV projections occurred, and the relatively small numbers of neurons projecting from AEV to the FAES would seem insufficient to provide visual activation across the entire territory of the FAES. However, it is possible that AEV-FAES projections become more highly branched in the early deaf, although this particular data is not available. Such increased axonal branches and terminals and their distribution area should be accompanied by increases in neuronal soma size to support the additional connections. However, the size of AEV neurons that project to FAES in hearing and in early-deaf animals did not change significantly across treatment groups. These observations, albeit indirect, do not support the notion that increased axonal sprouting from AEV could account for the territorial takeover of a deafened FAES.

It should be noted that the experimental conditions that induce crossmodal plasticity in the present experiment are not exactly the same as for the investigation that proposed the territorial expansion hypothesis (Rauschecker and Korte, 1993). While the present study involved complete deafening where the cochlear hair cells are ototoxically destroyed, the earlier studies employed visual deprivation where the functional integrity of the retina or optic nerves were not compromised. The visual deprivation studies did demonstrate that many of the auditory receptive field properties in the reorganized area were similar and receptive fields of neurons of both the FAES and reorganized AEV exhibited the same

spatial refinements (Rauschecker and Korte, 1993). Hence, the conclusion hypothesizing the territorial expansion mechanism was logical. However, for the area of the FAES to expand into and replace the AEV, the functionally reorganized AEV must then entirely redistribute its *outputs* so that the functional and behavioral role of the region would be changed to then subserve auditory localization. To our knowledge, however, such information is not available in blind/visually deprived preparations. It might also be suggested that deafness could induce connectional changes to AEV that were not examined by the present experimental design. Although a logical possibility, such an effect of deafness on non-auditory cortical regions would also imply that the connectivity of the entire brain would also change. However, numerous recent studies of deafness have failed to identify novel auditory or non-auditory cortical or thalamic projection sources to any of the cortical areas examined (Meredith and Allman, 2012; Kok et al., 2013; Chabot et al., 2015; Wong et al., 2015; Butler et al., 2016b).

These collective observations strongly indicate that deafness-induced reorganization of the FAES is unlikely to be attributable to the territorial expansion of the neighboring AEV as depicted in Figure 10. Therefore, territorial expansion does not provide a basic mechanism for crossmodal plasticity in general. Instead, a recent study comparing the afferent inputs of the early-deaf FAES with hearing controls identified that the unmasking/reweighting of existing connections broadly underlies the crossmodal phenomenon (Meredith et al., 2016) as represented in Figure 10, which is consistent with careful examinations of other crossmodally reorganized regions following early deafness (Meredith and Allman, 2012; Kok et al. 2013; Wong et al., 2015; Chabot et al., 2015; Butler et al., 2016). Further support for this mechanism is provided by the observation that synaptic connections are significantly increased in the FAES of early-deaf cats (Clemo et al., 2016), specifically in the supragranular layers which are the same layers that normally receive inputs from crossmodal (visual, somatosensory) areas (Clemo et al., 2008; 2016). In this way, it seems likely that crossmodal plasticity can reorganize a region such that it functionally resembles an adjoining region without being subsumed within the latter.

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Figure 1.

In normal development, cortical representations of different sensory modalities "A" and "B" occupy distinct and adjacent neural territories. However, if early loss of sensory modality "A" occurs, the Expansion Hypothesis predicts that area "B" will expand (thin arrows) to overlie the region vacated by the deprived sensory modality "A" (ghosted as grey).

Figure 2.

Summary of crossmodal plasticity in the early-deaf FAES. In hearing cats (top panel), neurons in the FAES (grey-shaded areas on serial coronal sections) largely respond to auditory (A) stimulation as indicated by the reconstructions of recording penetrations (vertical black lines; hash marks indicate location of a neuron whose sensory properties were examined). However, in early-deaf cats (bottom two panels) neurons in the FAES no longer encode auditory signals, but now respond largely to visual (V) or, to a lesser extent, somatosensory (S) stimulation (U = unresponsive). Note that early deafness resulted in a dramatic increase in the proportion of neurons that responded to visual stimulation, where the raster/histogram (lower right) displays the response of a FAES single-unit in an early-deaf animal (ramp indicates the passage of a visual bar of light across the receptive field; in the raster each dot=1 action potential; each row indicates responses to a single trial; the

histogram summarizes the neuron's activity in 10ms time-bins and clearly indicates a response to visual stimulation). The schematic of the brain (top right) shows the location of the FAES (grey area at arrow) and the vertical lines indicate the approximate A-P levels from which the coronal sections were taken. Hearing data (top panel) replotted from Meredith and Allman, 2009; early-deaf single-unit data (bottom panels) derived from Meredith et al. (2011) demonstrates the crossmodal reorganization of the same early-deaf cats (#BDA65; #BDA68) whose FAES connectivity is examined in the present study.

Figure 3.

Two examples of auditory brainstem response (ABR) evoked by stimulating (click, 90dB SPL) in the right ear (RE) or left ear (LE) of an early-deaf cat (case #BDA65-top; #BDA68-bottom). Each trace represents the averaged responses from 2000 trials at high-gain that fail to demonstrate the characteristic, multi-peaked ABR waveform that normally occurs over a period of ~5 ms following acoustic stimulation in hearing animals.

Figure 4.

Tracer injection sites for early-deaf FAES (top) and for AEV (bottom). The lateral view of the cat cortical hemisphere (grey, left) indicates the locations of areas FAES and AEV (at arrows), and the thick vertical line represents the approximate level ($\sim$ AP +9.0) from which the coronal sections (at right) were derived. Each coronal section shows the grey-white border (thin black line), some of the cortical regions near the AEV and FAES (see Table 1 for list of abbreviations), and the extent of the biotinylated dextran amine tracer (BDA) injection site (filled black area) for each animal (case number indicated by #).

Figure 5. Examples of retrogradely labeled neurons from Biotinylated Dextran Amine (BDA) tracer injection into visual AEV (A-left) or early-deaf FAES (B-right). In “A” the BDA-filled pyramidal neuron was located within the ALLS (case BDA23); in panel ‘B’ the labeled pyramidal neuron was located in area DZ (case BDA65). Scale bars = 50 μ m.

Figure 6.

Cortical projections to early-deaf FAES (A-top) or to AEV (B-bottom) are displayed on serially arranged (anterior-left; approximate AP-level listed across the bottom) coronal sections through cat (case BDA7-top; case BDAW-87-bottom) cerebral hemisphere. Displayed on the coronal sections are grey-white border (thin black lines), injection site (filled areas shaded grey), retrogradely labeled neurons (1 dot = 1 labeled neuron), and cortical functional subdivisions (light grey abbreviations; see Table 1 for list of abbreviations). Note that in these cases the projections to AEV largely avoided the auditory areas A1, A2, DZ, PAF and IN while those to early-deaf FAES did not.

Figure 7.

Comparison of visual cortical projection sources to AEV (grey bars) with those projecting to early-deaf FAES (black bars). Each bar represents the average proportion (percent $\pm$ sd) of the total ipsilateral cortical projection to AEV or to visually reorganized FAES. One area, the ALLS, exhibits similar connectivity with both AEV and early-deaf FAES. However, numerous other visual cortical areas (PS, VLS, AMLS, PMLS, PLLS) show significantly (“*,” $p < 0.05$, Wilcoxon) stronger projections to AEV than to early-deaf FAES. FAES connections from AEV could not be compared since AEV connections with itself could not be assessed (grey-X). Lower level visual cortical areas 17, 18, and 19 had only minimal connectivity with either AEV or early-deaf FAES. See Table 1 for list of abbreviations.

Figure 8.

Comparison of auditory cortical projection sources to AEV (grey bars) with those projecting to early-deaf FAES (black bars). Each bar represents the average proportion (percent $\pm$ sd; dots on error bars indicate value from individual case) of the total ipsilateral cortical projection. Projections to FAES from auditory areas AAF, A1 and DZ were significantly (“*”; Wilcoxon $p < 0.05$) greater than those to area AEV, while those from IN were significantly less. Early-deaf FAES connections to itself could not be assessed (black-X), hence no comparisons with FAES projections to AEV could be made. Scale on y-axis same as in Figure 5 for ease of comparison. See Table 1 for list of Abbreviations.

Figure 9.

Summary of the pattern of all corticocortical projections to visual area AEV (A-left) or to visually-reorganized FAES (B-right). These radial-plots depict the percent of the total ipsilateral corticocortical projection to the target area (scale = 20%; projections <1% of total not plotted) where each bar represents the mean percentage ($\pm$ se) of the projection proportion from the area designated at the edge of the column (abbreviations defined in Table 1 of abbreviations). As indicated in the key, the dark bars represent projections from visual cortical areas; medium gray bars from auditory areas; light grey bars from somatosensory areas. Note that the pattern of projections to visual AEV is dominated by inputs from visual areas (such as AMLS, ALLS, PLLS) and few inputs are present from auditory cortical sources (such as AAF, A1, A2 and DZ) while the opposite is observed for the early-deaf FAES. Hence, the pattern of cortical inputs to the two areas is not similar. See Table 1 for list of Abbreviations.

Figure 10.

After normal development, cortical representations of different sensory modalities “A” and “B” occupy distinct and adjacent neural territories (top). The present study shows that, if early loss of sensory modality “A” occurs, the results do not (large X) support the Expansion Hypothesis of crossmodal plasticity. Instead, sensory loss leads to the sensory reorganization of area “A” where function, connectivity and architectonics remain distinct from neighboring area “B.”

Table 1

List of Abbreviations

Abbreviation	Full name
Area A1	Primary auditory cortex (core auditory cortex)
Area A2	Second auditory cortex
Area 1	Primary somatosensory cortex
Area 3	
Area 3a	
Area 3b	
Area 4s	
Area 4f	
Area 5	
Area 5b	
Area 6a	
Area 6i	
Area 7	Parietal cortex
Area 7m	Parietal cortex, medial
Area 17	Primary visual cortex
Area 18	Secondary visual cortex
Area 19	Third visual cortex
Area 20a	
Area 20b	
Area 21a	
Area 21b	
Area 35	
Area 36	
AAF	Anterior Auditory Field (core auditory cortex)
AEV	Anterior Ectosylvian Visual area
AID	Agranular Insular-dorsal
AIV	Agranular Insular-ventral
ALG	Anterior Lateral gyrus visual area
ALLS	Anterolateral Lateral Suprasylvian visual area
AMLS	Anteromedial Lateral Suprasylvian visual area
CgA	Cingulate gyrus, anterior
CgP	Cingulate gyrus, posterior
CVa	Cingulate visual area
DLS	Dorsal Lateral Suprasylvian visual area
dPE	Dorsal Posterior Ectosylvian auditory area
DZ	Dorsal Zone of auditory cortex
FAES	Auditory field of the Anterior Ectosylvian Sulcus
G	Primary gustatory cortex
GI	Granular insular area

<u>Abbreviation</u>	<u>Full name</u>
IN	Insular auditory area
IL	Infra Limbic area
iPE	Intermediate Posterior Ectosylvian auditory area
ME	Medial Entorhinal area
MZ	Multisensory zone of rostral suprasylvian sulcus
PI	Parainsular area
PL	Prelimbic area
PLLS	Posterolateral Lateral Suprasylvian visual area
PMLS	Posteromedial Lateral Suprasylvian visual area
PS	Posterior Suprasylvian visual area
RS	Retrosplenial area
S2	Second somatosensory cortex
S2m	Second somatosensory cortex, medial
S3	Third somatosensory cortex
S4	Fourth somatosensory cortex
S5	Fifth somatosensory cortex
SVA	Splenial Visual Area
TE	Temporal auditory area
VAF	Ventral Auditory Field
VLS	Ventral Lateral Suprasylvian visual area
vPAF	Ventral Posterior auditory field
vPE	Ventral Posterior Ectosylvian auditory field