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Multisensory dysfunction accompanies crossmodal plasticity following adult hearing impairment

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Abstract

Until now, cortical crossmodal plasticity has largely been regarded as the effect of early and complete sensory loss. Recently, massive crossmodal cortical reorganization was demonstrated to result from profound hearing loss in adult ferrets (Allman et al., 2009a). Moderate adult hearing loss, on the other hand, induced not just crossmodal reorganization, but also merged new crossmodal inputs with residual auditory function to generate multisensory neurons. Because multisensory convergence can lead to dramatic levels of response integration when stimuli from more than one modality are present (and thereby potentially interfere with residual auditory processing), the present investigation sought to evaluate the multisensory properties of auditory cortical neurons in partially deafened adult ferrets. When compared with hearing controls, partially-deaf animals revealed elevated spontaneous levels and a dramatic increase (~2 times) in the proportion of multisensory cortical neurons, but few of which showed multisensory integration. Moreover, a large proportion (68%) of neurons with somatosensory and/or visual inputs was vigorously active in core auditory cortex in the absence of auditory stimulation. Collectively, these results not only demonstrate multisensory dysfunction in core auditory cortical neurons from hearing impaired adults but also reveal a potential cortical substrate for maladaptive perceptual effects such as tinnitus.

Keywords

aging; crossmodal plasticity; hearing loss; deafness; tinnitus; cortex

1.0

Historically, cortical crossmodal plasticity has been observed and characterized in individuals or animals that experienced profound sensory loss early in life (e.g., Bavalier and Neville, 2002; Lambertz et al. 2005; Auer et al. 2007; Frasnelli et al. 2011). In these

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CONFLICT OF INTEREST:

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cases, the neural representation of the lost sensory modality is replaced by inputs from the remaining sensory systems. This expanded neural representation can lead to perceptual and behavioral improvements in the remaining senses, termed ‘adaptive’ or ‘compensatory plasticity’ which have been reported for early-blind or early-deaf humans (e.g., Sadato et al. 1996; Roder et al. 2002; Merabet and Pascual-Leone, 2010; Sathian and Stilla, 2010;). Experimental models of compensatory plasticity have involved cats visually deprived from birth (Rauschecker and Korte, 1993; Korte and Rauschecker, 1993) as well as congenitally deaf cats (Lomber et al. 2010; 2011). Findings from those studies were important because recordings or deactivations could be made in the affected neural areas that correlated neuronal activity with the functional effects of the replacement sensory modality. The classic studies by Rauschecker and Korte (1993) showed auditory spatial localization was improved in visually-deprived cats, and that neurons recorded from a visual cortical region exhibited sharpened auditory spatial tuning. In addition, selective deactivation of crossmodally reorganized cortex resulted in the loss of specific compensatory functions (Lomber et al. 2010; 2011), demonstrating that the effects of crossmodal plasticity are not global but region-specific. Furthermore, region-specific compensatory effects were found to represent tasks or features common to both the lost and replacement modality (Meredith et al. 2011). These studies of the neural basis for compensatory plasticity, however, occurred in the context of profound sensory loss early in life. In contrast, few crossmodal cortical effects have been reported in relation to late-blindness or late-deafness even though large numbers of adults suffer from these forms of sensory loss.

A recent study showed that ferrets, profoundly deafened as adults, demonstrate robust crossmodal reorganization of their auditory cortices (Allman et al. 2009a). Although the complete loss of a particular sensory modality provides an experimentally simple model to examine crossmodal plasticity, partial sensory loss is a far more common neurological phenomenon. In fact, approximately 16% of adults in the USA experience some measure of hearing loss (Mitchell, 2006; Argawal et al. 2008). With such frequency of occurrence, adult hearing impairment represents one of the most prevalent neurological disorders. Findings from studies of adult-onset hearing impairment have been interpreted by some as indications of crossmodal plasticity (Blamey et al. 1996; Lee et al. 2003), especially because deafened adults who delay cochlear implantation often have less success with the devices than do those who receive immediate intervention (Proops et al. 1999). However, hearing-impairment also affects spiral ganglion cell survival (Leake et al., 1991) and substantially changes neuronal firing rates and inhibition throughout the auditory pathway (Kaltenbach et al. 2000; Salvi et al. 2000; Norena and Eggermont, 2003; Seki and Eggermont, 2003; Kotak et al. 2005; Ma et al. 2006; Mulders and Robertson, 2009; Mulders et al. 2011), and it is not known if these firing rate changes, or crossmodal plasticity, underlie the clinical observations.

In the context of early sensory loss, crossmodal plasticity results in neurons that are responsive to the replacement modality (e.g., Rauschecker and Korte, 1993; Meredith and Lomber, 2011) but are obviously insensitive to stimulation provided through the lost sensory modality. Therefore, many of the reorganized neurons are unisensory in nature. In contrast, partial sensory loss leads to a situation where a region may retain some residual input/function in the damaged modality while also receiving convergent information from the intact sensory systems. This condition suggests that partial sensory loss could result not in sensory replacement, but multisensory convergence. In fact, a recent study of partially-deafened adult ferrets demonstrated that the most prevalent form of auditory cortical neuron was multisensory (Keniston et al. 2011). It is well known that multisensory convergence leads to multisensory integration (for review, see Stein and Meredith, 1993), where the neuronal response to stimulation in one sensory modality is significantly altered by stimulation in another modality. Multisensory integration can enhance a neuron’s response

by 1200% or depress it to zero, depending on the physical parameters of stimuli involved (Meredith and Stein, 1986). Multisensory integration has also been observed in cortical multisensory neurons in a variety of species. However, the influence of multisensory integration on residual auditory cortical processing in partially-deafened subjects is not known, nor have the full criteria for multisensory integration been applied to crossmodal studies of hearing loss. Although a subset of core auditory cortical neurons in normal hearing animals tend to exhibit multisensory response suppression (Bizley et al. 2007), it is unknown if hearing impairment might contribute to or diminish this established effect. Therefore, the present experiment was designed to examine the multisensory properties of neurons in core auditory cortex that has been crossmodally reorganized by adult partial hearing loss.

2.0 EXPERIMENTAL PROCEDURES

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), and approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

2.1 Ototoxic Procedures

In 5 ferrets aged 189–240 days postnatal (DPN; see Table 1) a combination of kanamycin (100 mg/kg, subcutaneous) and ethacrynic acid (25 mg/kg, i.v.) was used to systemically induce a partial lesion to the cochleae. Nine weeks after the ototoxic procedure, the level of hearing impairment was assessed for each ear separately, using auditory brainstem responses (ABRs). The auditory stimulus was a calibrated click (2000 trials each, 0.1 ms square-wave click, rarefaction), delivered through a speaker positioned in front of one ear. Subdermal recording leads were inserted over the right and left mastoid processes, at mid-cranium and at mid-back. Evoked electrical activity was signal averaged and threshold response levels were determined using a descending (5–10 dB increments) sequence of sound intensity for each ear of each animal. Bilateral ABRs were also tested on hearing animals ($n=5$; threshold ~15dB SPL). The ABR results are shown in Table 1. Once an animal's auditory threshold was established, it was prepared for recording.

2.2 Surgical Procedures

Each ferret (hearing impaired and controls) was surgically prepared 2–3 days before electrophysiological recording. Under aseptic conditions and pentobarbital anesthesia (40 mg/kg, i.p.), a craniotomy was opened to expose the left auditory cortices. A stainless steel head-support device was implanted over the opening and the scalp was closed around the implant with standard postoperative care provided.

2.3 Electrophysiological Recordings

Approximately 10 weeks after the ototoxic treatment, the sensory responses of the auditory cortices were sampled; hearing ferrets of a similar age were also examined (see Table 1). For recording, the animal was anesthetized (35 mg/kg Ketamine; 2 mg/kg Acepromazine) and the implanted well was secured to a supporting bar. The animal was intubated through the mouth and ventilated with expired CO₂ monitored. Fluids and supplemental anesthetics (4 mg/kg/h Ketamine; 0.5 mg/kg/h Acepromazine) were continuously administered using an infusion pump. Because it was essential that the animal's body remained motionless during the sensory tests (described below), a muscle relaxant (Pancuronium bromide 0.3 mg/kg i.p. induction) was also infused (0.2 mg/kg/h). Heart rate and body temperature were continuously monitored and a heating pad was used to maintain temperature.

The implanted recording well was opened and the 32-channel probe (~1 M Ω , 1×32×5mm, #100–413, NeuroNexus Technologies, Ann Arbor, MI) was positioned in auditory cortex to ~2.0 mm depth using gyral/sulcal landmarks (Bizley et al., 2005). At each recording site, neurons were initially screened for spontaneous activity or their responses to an extensive battery of manually-presented stimuli that included auditory (clicks, claps, whistles and hisses), visual (flashed or moving light or dark stimuli), and somatosensory (air puffs, brush strokes and taps, manual pressure and joint manipulation, and taps using calibrated Semmes-Weinstein filaments) stimuli.

Following manual assessment of sensory responsiveness, additional, quantitative sensory tests were performed that consisted of computer-triggered auditory, visual and somatosensory stimuli, presented alone and in combination. Free-field auditory cues were electronically-generated white-noise bursts (100 ms) from a speaker 44 cm from the head in contralateral space (45° azimuth/ 0° elevation). For hearing impaired animals, auditory stimulation intensity was 30–50 dB (avg. 38 dB $\pm$ 0.5 s.e.m.) above their hearing threshold. Auditory stimuli for hearing animals ranged from 35–55 dB (avg. 51 dB $\pm$ 0.35 s.e.m.) above their hearing threshold. Visual cues were projected onto a translucent hemisphere (92 cm diameter) with programmed control of movement direction, velocity and amplitude. Because it often was not possible to map visual receptive fields, a standard visual stimulus was employed: a vertically-oriented bar of light (~2×10°), transited 30° from nasal-to-temporal in the upper quadrant of contralateral visual space (see also Allman et al. 2008). An electronically-driven, modified shaker (Ling, 102A), with independently programmable amplitude and velocity settings delivered focal somatosensory stimulation through a calibrated (1 g) 5 cm nylon filament. This stimulus was placed within the manually mapped somatosensory receptive field; if no receptive field was apparent, the stimulus was placed on the contralateral side of the face, which was the location of highest probability observed in profoundly deaf ferrets (Allman et al. 2009a). To control for the possible acoustic effects of the moving tactile stimulus, each set of somatosensory trials was followed by a sham test in which the device remained in place, but with the filament disengaged from the body surface. Accordingly, neurons affected by the sham tactile trials were designated as auditory. During the combined-modality presentations, the visual stimulation onset preceded auditory and somatosensory stimulation onset by 50 ms, to roughly compensate for the response latency differences of the different modalities (Allman et al. 2008; Wallace et al. 1992). Each stimulus presentation was separated by 3–7 s, and each condition was presented 50 times. Because hearing loss disrupts the cochleotopy of auditory cortex (Schwaber et al., 1993; Fallon et al., 2009) and eliminates or diminishes the sensitivity of individual neuronal frequency tuning curves, no attempt was made to examine these auditory-specific properties.

2.4 Data Analysis

For single-unit recording, neuronal responses were digitized at 25 kHz using a Tucker Davis neurophysiology workstation (System III, Alachua, FL) and stored on a PC for later analysis. Individual neuronal waveforms were sorted offline using an automated Bayesian sort-routine (v. 2.10, OpenSorter, Tucker Davis) to identify and group waveforms that represent distinct, single units. For each neuronal template, responses to stimuli were identified after the method of (Bell et al. 2001): response onset was defined as the point which activity level exceeded 3 standard deviations above median spontaneous activity (measured from the 500 ms period before stimulus onset), with a minimum of 15 ms of activity sustained at that level; response offset was defined as activity that dropped below the sustained response level and remained below it for 15 ms; response duration was defined as the period between the response onset and offset. The time span between onset and offset was used to calculate the spike counts evoked by sensory stimulation and, in this manner, a mean (and standard deviation) spike count was determined for somatosensory, auditory, visual and combined-

modality stimulation for each neuron. An activity of >0.5 mean spikes per trial in response to at least one stimulus permutation was required to qualify as a suprathreshold response. To avoid well-known floor effects on measures of multisensory integration, neurons with low response levels (<1.0 spike/trial) were not included in the population analysis of multisensory effects. Finally, a paired t-test was used to assess if each separate-modality response (auditory alone, visual alone, tactile alone) differed significantly from the combined condition (auditory-visual together, auditory-tactile together, auditory-visual-tactile together), according to established criteria that define neuronal multisensory integration (Meredith and Stein, 1986). Unisensory neurons were identified as those which were activated or influenced by only one sensory modality. Multisensory neurons activated by two different sensory modalities were defined as bimodal; those activated by three were identified as trimodal neurons. Multisensory neurons activated by one modality but whose response could be modulated (suppressed or facilitated) by a second modality that was ineffective alone were categorized as subthreshold neurons (see Allman et al. 2009b). These measures of sensory responsiveness were tabulated along with the neuron's position and depth within a data spreadsheet for analysis.

At the end of an experiment, the animal was euthanized and the brain was fixed, blocked and serially sectioned ($50\text{ }\mu\text{m}$). The sections were processed and stained (Cresyl violet) using standard histological procedures to reconstruct the recording penetrations. By tracing the outline of layer 4, the supra-, infra- and granular locations of individual recording sites could be determined. However, distinctions between layers 2–3 and layers 5–6 could not be visualized with this counterstain. The sulcal/gyral patterns for ferret auditory cortex depicted in Figure 1 (inset) were identified by Bizley et al. (2005). Because the borders of A1/AAF and the adjoining visual/somatosensory fields (e.g., anterolateral and posterolateral suprasylvian visual areas: Manger et al. 2008; lateral rostral suprasylvian somatosensory area, Keniston et al. 2008) have not been defined, only those neurons histologically verified within the gyral aspects of the cortical mantle were included in the present study.

3.0 RESULTS

In normal hearing adult ferrets ($n=5$; hearing threshold $\sim 15\text{ dB SPL}$), 15 recording penetrations identified a total of 311 neurons within A1/AAF cortical regions. Each of these neurons was subjected to the same series of sensory tests that included auditory, somatosensory and visual stimulation alone as well as in combination (auditory-visual, auditory-somatosensory and auditory-visual-somatosensory). Depending on their responses to these quantitative sensory assessments, neurons were classified as unisensory or multisensory (defined in Methods). All but one sensory neuron was activated by auditory stimulation, and most (65%; 203/311) were exclusively influenced by an auditory cue. An example of a unisensory auditory neuron is provided in Figure 2A, where its vigorous response to auditory stimulation is not significantly affected by the concurrent presence of a tactile or visual cue, nor is the neuron's spiking activity affected by these non-auditory stimuli when presented alone. In addition, numerous (34%; $n=107/311$) neurons were also activated (i.e., bimodal, trimodal) or influenced (i.e., subthreshold multisensory) by tactile or visual stimulation. Such multisensory neurons are illustrated in Figures 3A–B and their incidence in the core auditory cortex of hearing ferrets is summarized in Figure 4A.

The adult, ototoxically-treated ferrets ($n=5$) revealed elevated hearing thresholds that averaged $\sim 47\text{ dB SPL}$ (range 35–55 dB SPL; see Table 1). In these animals, 10 recording penetrations yielded a total of 284 neurons that were histologically verified within A1/AAF, as depicted in Figure 1. Each of these neurons was presented the same set of quantitative sensory assessments that included auditory, somatosensory and visual stimulation presented alone as well as in combination (auditory-visual, auditory-somatosensory and auditory-

visual-somatosensory). Almost all responsive neurons were activated by acoustic stimulation (99%; $n=281/284$). Many (31%; $89/284$) of these acoustically-responsive neurons were exclusively activated by auditory stimulation, and an example of a unisensory auditory neuron from a hearing impaired animal is provided in Figure 2B. However, most other neurons (68%; $n=193/284$) were either activated by another sensory modality (i.e., bimodal/trimodal) or were significantly influenced by the presence of a non-auditory stimulus (i.e., subthreshold multisensory). Examples of these forms of multisensory neurons are depicted in Figures 3C–D and their incidence in core auditory cortex of adult hearing-impaired ferrets is summarized in Figure 4. Within the different cortical layers, bimodal/trimodal multisensory neurons always represented the major neuron type regardless of their supra- (54%), infra- (51%) or granular (61%) location. Unisensory neurons showed similar proportions at supragranular (40%) and infragranular (38%) locations, and subthreshold multisensory neurons were consistently infrequent (~12%; range 6–16%) across all laminae. These laminar observations indicate that the crossmodal effects were distributed rather uniformly across the cortical layers.

3.1 Spontaneous activity

It is visually obvious from Figures 2 and 3 that the spontaneous levels of activity of core auditory cortical neurons in normal hearing animals were dramatically lower than that observed in the hearing impaired animals. The rasters are denser and the y-axis of response histograms are consistently higher for neuronal data from the treated animals, and these differences were apparent in many other response records as well. To statistically examine this effect, the data records for each sensory test for each neuron from normal hearing ($n=311$) and from hearing impaired animals ($n=284$) were examined. From these records, spontaneous spike counts were made during the 500 ms prior to the onset of stimulation, which were then normalized to 1 second so that the standard units of spikes/second were applicable. As illustrated in the bar graphs of Figure 5A, the average spontaneous activity observed in core auditory cortex of normal hearing ferrets was 14.88 spikes/sec (± 0.74 s.e.m.), while spontaneous activity in the same regions of the hearing impaired animals was 24.2 spikes/sec (± 1.1 s.e.m.) representing an average 63% increase in activity; these values were statistically different ($p<0.0001$, t-test).

3.2 Responses to Unisensory Stimuli

Because spontaneous activity in core auditory cortex was so dramatically increased by partial hearing loss, it seemed likely that the physiological mechanisms underlying this effect might also influence sensory response levels. Therefore, the spike counts elicited by sensory stimulation (tactile, visual) were compared between the samples from hearing impaired and normal hearing animals. For tactile stimulation, the same 1 g nylon filament stimulus was used for both the treatment and control groups. In hearing impaired animals, core auditory cortical neurons showed tactile responses that averaged 5.6 spikes/trial (± 0.57 s.e.m.), which was significantly ($p<0.001$, t-test) higher than in those from hearing controls (avg. = 2.6 spikes/trial ± 0.26 s.e.m.) (see Fig. 5B) and represented an average 115% response increase. All neurons classified as somatosensory were influenced by the tactile stimulus, but not by the subsequent sham trial in which the stimulator was activated but did not touch the body surface. In the visual modality, responses to visual stimulation in hearing impaired animals were also significantly ($p<0.001$, t-test) higher (avg. = 4.9 spikes/trial ± 0.62 s.e.m.) than their control counterparts (avg. = 1.85 spikes/trial ± 0.35 s.e.m.), representing an average 165% response increase. These sensory response comparisons are summarized in Figure 5B, which indicates that all forms of sensory-evoked spiking activity in A1/AAF neurons from hearing impaired animals were elevated above normal levels. To control for the effect of the elevated spontaneous activity itself, spontaneous levels were subtracted from the average response values depicted in 5B for both sets of stimulation and

treatment groups, but the differences between hearing-impaired and controls remained. This same trend for increased response levels was also observed for the hearing-impaired animals following acoustic stimulation, but these results could not be appropriately compared because different stimulation intensities were required for the different treatment groups.

3.3 Responses to Multisensory Stimuli

In both preparations, neurons responsive to non-auditory stimulation were almost always sensitive to acoustic cues as well, meaning that many neurons in normal hearing (34%) as well as hearing impaired (68%) animals were multisensory. With the presence of a large proportion of multisensory neurons in auditory cortices, how these neurons respond to multisensory stimulation may profoundly influence auditory processing and perception. Therefore, for core auditory neurons defined as multisensory, the activity evoked by an auditory stimulus was plotted against the response elicited by the combination of auditory-visual, auditory-tactile, and auditory-visual-tactile stimulation. For neurons from hearing animals, the effect of auditory versus auditory-visual stimulation showed a strong bias toward multisensory suppression, where 77% of multisensory neurons showed responses to the combined stimuli that were lower than those elicited by the acoustic cue alone (see Fig. 6A). As a population, responses to the auditory-visual combination was significantly ($p < 0.001$, t-test) lower than that evoked by auditory stimulation alone and a line of best fit through the data fell well below the line of unity. The same multisensory suppressive trend was also observed for neurons sensitive to auditory-tactile, and auditory-visual-tactile stimulation, where 81% and 78% of the sample, respectively, plotted below the line of unity. In contrast, as shown in Figure 6B, responses of multisensory neurons in hearing impaired animals to the same stimulus combinations did not elicit the same suppressive trends. Instead, for each stimulation condition, multisensory neurons showed nearly equal proportions with facilitative or suppressive changes: visual-auditory neurons 54:46%; visual-tactile 44:56%; auditory-visual-tactile 51:49%, respectively. Accordingly, the line of best fit through the data for each of the stimulation combinations plotted very near the line of unity as if the influence of non-auditory stimulation was attenuated in both the negative and positive directions.

In addition to the sign (positive or negative) of multisensory response changes, the result of multisensory processing can also be assessed in terms of response magnitude. For each multisensory neuron, the response change induced by combined-modality stimulation was compared with that of the most effective single-modality stimulus for each multisensory neuron (after Meredith and Stein, 1986). Figure 7A summarizes these measures for multisensory neurons as a proportion of all sensory neurons tested. In this way, the greater incidence (~2 times) of multisensory neurons identified in partially-deafened animals (gray bars) than in normal hearing animals (black bars) was clearly evident. Overall response changes induced by multisensory stimulation exhibited nearly the same range (from 90% to -60%) for both hearing impaired and normal hearing populations. However, the distribution of the magnitude of multisensory response changes centered near 0% (avg. $0.15\% \pm 1.8$ s.e.m.) for multisensory neurons from hearing impaired animals, but shifted toward the negative direction for normal hearing animals (avg. $-11\% \pm 2.7$ s.e.m.), which was statistically significant (Wilcoxon Rank Sum Test; $P < 0.001$). In fact, the most prevalent response levels observed in hearing impaired animals occurred between 20 and -20% (representing 60% of multisensory neurons) while most (70%) occurred between 0 and -40% in the normal hearing ferrets. Thus, the magnitude of responses to combined-modality stimulation generally showed little change for neurons in partially-deafened ferrets, but tended to show suppression in normal hearing animals (see also, Bizley et al. 2007).

If the magnitude of response change elicited by multisensory stimulation meets specific statistical criteria (see Methods), the result is defined as multisensory *integration* (Meredith

and Stein, 1986). To examine the effect of partial-deafness on multisensory integration in core auditory neurons, responses that satisfied the required statistical criteria for integration were presented in Figure 7B. Those response changes included in Figure 7A that did not meet the criteria for multisensory integration most often represented the lowest magnitude responses (e.g., mostly between 20 and -20%) are absent in Figure 7B. In this comparison, of the overall sample of multisensory neurons encountered in each treatment group, proportionally more neurons from normal hearing animals showed integrative multisensory responses. As summarized in Figure 7C, of the multisensory neurons identified in normal hearing animals (34% of entire sample), approximately two-thirds (64%) of them showed significant levels of multisensory integration. In contrast, of the multisensory neurons sampled from hearing impaired animals (68% of entire sample), far less than half (30%) met the statistical criteria for multisensory response integration. Thus, the proportion of neurons that demonstrated multisensory integration was significantly reduced in hearing impaired animals versus controls (chi-square test, $p < 0.0001$). Furthermore, the integrative values observed in the control and the hearing-impaired animals were statistically different (Wilcoxon Rank Sum Test; $P < 0.022$). With regards to enhanced or depressed forms of multisensory integration, comparatively few core auditory cortical neurons exhibited significant response enhancement in either the hearing impaired (14%; range=18–65%) or normal hearing (10%; range 20–90%) groups. In contrast, significant response depression was observed in only a small proportion of multisensory neurons from hearing impaired animals (21%; range=-14 to -60%), while occurring in a majority of multisensory neurons from the hearing control group (56%; range=-9 to -55%). Thus, the proportions of neurons demonstrating multisensory response depression was significantly reduced in hearing impaired animals versus controls (chi-square test, $p < 0.002$). These results indicate that multisensory integration, specifically multisensory response depression, is strongly attenuated in multisensory neurons of core auditory cortex in adult hearing-impaired ferrets.

4.0 DISCUSSION

The present study extends the findings of crossmodal plasticity in auditory cortex following hearing loss in adult animals. As with adult animals with profound hearing deficits (>90 dB SPL threshold; Allman et al. 2009a), adult ferrets with moderate hearing loss (35–55 dB SPL threshold) revealed a profound increase in the proportion of neurons responsive to non-auditory stimuli. Furthermore, these crossmodal effects were rather uniformly distributed across the cortical layers. However, unlike animals with profound hearing loss, residual auditory function was present in the hearing impaired animals, resulting in a large population of neurons that were responsive to both auditory and non-auditory inputs. In other words, sub-total hearing loss results not in the substitution of crossmodal inputs, but in increased convergence of non-auditory with auditory inputs. The increased presence of multisensory neurons in A1/AAF cortex enhances the likelihood that non-auditory inputs influence neurons there, whereby the non-auditory responses can dramatically affect auditory processing through multisensory integration. While crossmodal substitution has long been recognized as a potential confound for auditory processing in the hearing impaired, the present study demonstrates that the presence of non-auditory inputs does not preclude auditory function. In fact, 99% of the core auditory neurons in the hearing impaired animals responded to acoustical stimulation. However, in these partially deafened animals, the most prevalent form of auditory cortical neuron showed multisensory convergence (68%), indicating that crossmodal inputs interact with, rather than replace, residual auditory function.

Given that multisensory integration can profoundly enhance or depress neural responses (Meredith and Stein, 1986), it is clearly important to assess the influence of multisensory integration on auditory processing in hearing impaired individuals. It is well known that

hearing loss results in elevated neural activity throughout the auditory pathway (Kaltenbach et al. 2000; Salvi et al. 2000; Norena and Eggermont, 2003; Seki and Eggermont, 2003; Kotak et al. 2005; Ma et al. 2006; Mulders and Robertson, 2009; Mulders et al. 2011). Therefore, because the neurons are firing higher on the rate-response curve, it might be expected that examples of multisensory enhancement would be reduced (due to inverse effectiveness, Meredith and Stein, 1986) in magnitude and/or number. The present results show comparatively few (~14%) auditory cortical neurons from hearing impaired animals exhibited multisensory enhancement that ranged from 18–65% increases in firing rates. However, a similarly low proportion (10%) exhibited enhancement and spanned a similar range (20–90%) in the hearing controls. With such low levels of incidence and effect, it is difficult to discern a relationship between partial hearing loss and multisensory enhancement. On the other hand, conditions of elevated neuronal activity should favor the detection of inhibitory multisensory interactions which, incidentally, are the dominant form of multisensory effect in auditory cortex of normal hearing animals (Bizley et al. 2007). In fact, in normal hearing animals statistically significant levels of multisensory response depression were observed in 56% of the multisensory neurons from normal hearing animals (present study). In contrast, in animals with hearing loss only 21% of multisensory neurons exhibited suppressive interactions. Therefore, by several measures, these data indicate that multisensory neurons in auditory cortex of hearing impaired animals exhibit a dysfunctional form of multisensory integration.

The diminution of cortical multisensory suppression in partially-deafened adults is consistent with numerous systemic changes following hearing impairment that may result from alterations in excitatory-inhibitory balance, homeostatic plasticity, or both. As already mentioned, hearing loss is accompanied by increased levels of spontaneous activity in the dorsal cochlear nucleus (Kaltenbach et al. 1998; Brozoski et al. 2002; Chang et al. 2002; Shore et al. 2008; Finlayson and Kaltenbach, 2009) and inferior colliculus (Vale and Sanes, 2000; Ma et al. 2006; Bauer et al. 2008; Dong et al. 2010) where reductions in synaptic inhibition involving glycinergic and GABAergic forms have been observed (Brozoski et al. 2002; Dong et al. 2010; Wang et al. 2009). Furthermore, brain stem alterations in excitation-inhibition are exacerbated by transmission changes in auditory cortex where synaptic excitation is strengthened, as indicated by larger and longer EPSPs and increased postsynaptic glutamate sensitivity, while intrinsic synaptic inhibition is decreased, as evidenced by reduction of GABA_A-mediated IPSP amplitude (Kotak et al. 2005) and inhibitory synaptic function (Takesian et al. 2009; Xu et al. 2010). These phenomena are also likely to recruit homeostatic mechanisms that adjust neuronal firing properties. Specifically, changes in network activity dynamically adjust the amplitude of excitatory (Turrigiano et al., 1998) and inhibitory (Killman et al., 2002) postsynaptic currents. In fact, reduced neural activity has been demonstrated to scale up excitatory synapses onto cortical pyramidal neurons while reducing the excitation of some forms of inhibitory interneurons (Rutherford et al., 1998; Chang et al., 2010). Furthermore, reduced network activity also intensifies the intrinsic excitability of pyramidal neurons such that they exhibit increased firing levels to the same synaptic input (Desai et al., 1999). Collectively, these effects indicate that the homeostatic regulation of neuronal excitability (for review, see Turrigiano, 2011; Wenner, 2011) is also likely to occur in response to reductions in sensory driving following hearing loss or impairment. Therefore, it seems plausible that the system-wide changes in neuronal firing properties following hearing loss may not only account for the observed increases in non-auditory responsiveness, but also in the reduction of multisensory integration as well. Whether the specific reduction in multisensory depression in multisensory neurons from hearing-impaired animals is based on changes in ion channel conductance, synaptic scaling, or both, remains to be determined.

The mechanisms underlying cortical crossmodal plasticity have been of considerable speculative interest. In a review, Rauschecker (1995) summarized the known possibilities as involving either the unmasking of silent inputs, the stabilization of normally transient connections, the sprouting of new axons, or a combination thereof. These options were offered largely to explain the visual crossmodal effects that were apparent at that time. However, our recent observations of largely somatosensory reorganization of core auditory cortex in deaf adults (Allman et al., 2009a; present study) or juveniles (Meredith and Allman, 2012) do not readily conform to these earlier postulates. With regard to the possibility that hearing loss unmasks silent somatosensory inputs to core auditory cortex, such crossmodal inputs should be evident in the auditory cortex in normal hearing animals. Yet, in neither the ferret (Allman et al., 2009a; Meredith and Allman, 2012) nor the cat (Lee and Winer, 2008) is there sufficient connectivity from somatosensory cortical or thalamic structures to underlie the robust somatosensory reorganization of the core auditory area after hearing loss. Alternatively, if crossmodal plasticity was subserved by the preservation of transient connections, then core auditory connectivity should be different between the early hearing-impaired and the normal hearing animals. However, early hearing-impaired animals show essentially the same core auditory connectivity as their normal hearing counterparts (Meredith and Allman, 2012). Moreover, the preservation of transient connections cannot account for the somatosensory reorganization observed in adult-deafened ferrets (Allman et al., 2009a; present study). Finally, the possibility that axonal sprouting from existing somatosensory sources cannot account for the observed effects in core auditory cortex of adult deafened (Allman et al., 2009a) or hearing impaired ferrets (present study) because somatosensory responses have been observed there within 15 days of hearing loss (Allman et al., 2009a), which is a time period that is far too short to permit new axonal ingrowth even from the nearest somatosensory cortical region. Therefore, an alternate hypothesis for the mechanism underlying hearing-loss induced somatosensory reorganization has been proposed (Allman et al., 2009a; see also Levine, 1999a). As has been known for over a decade now, the auditory brainstem naturally receives crossmodal inputs from the somatosensory system at several critical nodes: the dorsal cochlear nucleus (Davis and Young, 1997; Kanold and Young, 2001; Kanold et al., 2011; Shore et al., 2000) as well as the inferior colliculus (Aitkin et al., 1981; Dehmel et al., 2008; El-Kashlan and Shore, 2004). In addition, hearing loss enhances the level of crossmodal somatosensory innervation of the dorsal cochlear nucleus (Shore et al., 2008). Therefore, Allman et al. (2009a) proposed that because cochlear damage results in enhanced somatosensory crossmodal plasticity in the cochlear nucleus, which is the first node in the ascending auditory projection, and any functional changes within that nucleus should be reflected throughout the entire auditory pathway, including cortex. This postulate is consistent with the representation of trigeminal and cervical somatosensory regions in deafened core auditory cortex. Furthermore, given the highly crossed nature of the ascending auditory projection, it is not surprising that a high proportion of crossmodal somatosensory receptive fields in auditory cortical neurons are bilateral (Allman et al., 2009a; Meredith and Lomber, 2011; Meredith and Allman, 2012). Thus, the brainstem theory of core auditory cortical crossmodal reorganization is supported by empirical observations from different published points of view. That said further investigation is necessary to reconcile the somatosensory and visual spheres of crossmodal influence that result from hearing loss.

Collectively, these observations reveal an auditory system that is substantially altered by partial hearing loss, resulting in increases in the incidence of multisensory convergence. However, such multisensory convergence appears to produce little integrative effect on residual auditory function. Indeed, although many multisensory neurons are generated (68% of core auditory cortical neurons from hearing impaired animals), examples of multisensory enhancement are sparse and the levels and incidence of multisensory depression are reduced. These data would suggest that the responses to combined-stimulation do not significantly

differ from the responses to effective unimodal stimulation. This notion is confirmed by the plots in Figure 6B which also show no statistically significant multisensory population effects on auditory responses. Because multisensory response depression is apparent on the population of core auditory cortical responses from normal hearing animals (Bizley et al., 2007; Figure 6A present study), this processing deficit must represent a form of multisensory dysfunction in hearing-impaired animals.

Even though the increase in multisensory neurons in auditory cortex of hearing-impaired animals was not paralleled by increases in multisensory integrative effects, it is important to recognize that the increased presence of non-auditory inputs alone generates neuronal firing in a majority of sampled neurons. In the hearing impaired animals, the response levels elicited by visual or tactile stimuli were significantly above those observed in normal hearing group, and a link has been suggested between elevated firing rates in the auditory system and hyperacusis (Nelson and Chen, 2004). More importantly, the increased non-auditory inputs and responses definitely subserve activity in the core auditory cortex that occurs *in the absence of acoustic stimulation*. Because electrical or magnetic activation of core auditory cortex leads to perception of sound (reviewed in Selimbeyoglu and Parvizi, 2010), the non-auditory activation of core auditory cortex described here could also underlie spurious perceptions of sound when none is present. Thus, the cortical substrate for tinnitus appears to be manifest in animals with hearing impairment (present study) or profound hearing loss (Allman et al., 2009a) as adults or juveniles (Meredith and Allman, 2012), as each of these studies documented the largely somatosensory crossmodal reorganization of core auditory cortex. These results are also consistent with the clinical literature, where approximately 68% of tinnitus patients can somatically modulate the psychoacoustic features of their tinnitus (Levine, 1999b; Levine et al., 2003), and indwelling (De Ridder et al., 2007) or transcranial magnetic (TMS; Meeus et al., 2009) stimulation of auditory cortex can dramatically reduce the perception of specific types of tinnitus. These collective observations suggest that maladaptive perceptual effects, such as tinnitus or hyperacusis, may result from the crossmodal reorganization of auditory cortex following hearing loss at any age. Given the likely brainstem origin for these crossmodal effects (Levine, 1999a; Norena and Eggermont, 2003; Ma et al., 2006; Dehmel et al., 2008; Shore, 2011; Shore et al., 2008; Allman et al., 2009a) a logical and significant challenge will be to determine if this plasticity can be blocked in its ascent to the cortex.

4.1 CONCLUSIONS

When compared with normal hearing controls, the auditory cortex of ferrets that incurred a partial hearing loss (35–55dB SPL thresholds) as adults showed an increase in spontaneous activity as well as in response levels to auditory, visual and somatosensory stimulation. In addition, partially deafened cortex revealed an increase in multisensory convergence (from 34% to 68% of neurons), but the increased proportions of multisensory neurons did not exhibit commensurate increases in multisensory integration. Instead, fewer instances of multisensory integration were observed, as evidenced by a broad reduction in the incidence and magnitude of multisensory depression. While such multisensory dysfunction is consistent with the system-wide excitatory/inhibitory imbalance known to accompany hearing loss, crossmodal reorganization of core auditory cortex appears to provide the cortical substrate underlying maladaptive perceptual disorders such as tinnitus.

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Highlights

Auditory cortical neurons in ferrets partially deafened as adults showed:

- Auditory responsiveness remained robust, albeit at higher thresholds
- Spontaneous and sensory response levels increased
- Crossmodal somatosensory, visual and multisensory innervation increased
- Incidence and magnitude of multisensory integration decreased
- Collectively, such non-auditory activation of core auditory cortex may underlie tinnitus

Figure 1.

Ferret cortex (inset) showing the core auditory areas of primary auditory (A1) and anterior auditory field (AAF) areas (redrawn from Bizley et al. 2005). Vertical lines indicate the approximate coronal levels from which the enlarged, serially-arranged (anterior=left) sections are derived. The tissue sections through core auditory cortex (dashed lines denote borders of A1 and AAF, at arrows) of adult hearing-impaired ferrets depict the location of layer 4 (thin parallel lines labeled 'IV'), thus indicating supragranular (above) and infragranular (below) locations. The coronal sections also summarize the recording sites and the sensory features of the recorded neurons. Each symbol (see key) along an electrode shaft (black line) indicates a recording site and its corresponding recorded sensory properties. Only neurons localized within the gyral aspects of A1/AAF were accepted in this study, although some non-included sites are depicted for completeness. Not every neuron is illustrated due to overlap.

A. Hearing

B. Hearing Impaired

Figure 2. Unisensory auditory neurons

(A) This core auditory neuron from a normal hearing adult hearing ferret was excited by an auditory stimulus (square-wave labeled ‘A-only’), but not by a visual (ramp labeled ‘V only’), tactile (ramp labeled ‘T only’) or visual-tactile combination (VT). In addition, spiking activity elicited by combinations involving auditory stimuli (AV, AT,) were not significantly different from that to auditory alone. (B) A unisensory core auditory neuron from an adult, hearing impaired ferret. This neuron was excited by an auditory stimulus, but not by a visual or tactile stimulus, or by their combination. In addition, spiking activity elicited by combinations of stimuli (AV, AT) was not significantly different from that to auditory alone. When compared with its counterpart in a normal, hearing animal, note the higher spontaneous activity of the neuron from the lesioned animal. Each raster represents spiking activity (1 dot=1spike) during 50 presentations (ordered from bottom to top) that is summarized (at bottom) by the peristimulus time histogram (10 ms time bin). These conventions are used in subsequent figures.

Figure 3.

Responses of multisensory neurons in A1/AAF of a hearing adult ferret (left column, A–B) and from a ferret with adult-onset hearing loss (right column, C–D). **(A)** Responses of a bimodal neuron that was reliably activated by an auditory stimulus (left; square wave labeled ‘A’) but weakly by a tactile stimulus (ramp labeled ‘T’), but not by a visual stimulus (ramp labeled ‘V’). In addition, the combination of auditory and tactile stimulation generated a response that was significantly lower than that elicited by the auditory stimulus alone. Thus, this neuron was not only bimodal, it exhibited multisensory response depression. **(B)** This neuron appeared to be a unisensory auditory neuron that was reliably excited by an auditory, but not by a visual or tactile stimulus. However, when the auditory and visual stimuli were presented together, a significant response reduction occurred that demonstrated the subthreshold multisensory nature of the neuron in the form of response suppression. **(C)** These recordings from an adult, hearing-impaired animal show the responses of a trimodal auditory-visual-tactile neuron that exhibited a modest response increase when the same stimuli were combined. **(D)** This bimodal neuron was activated by an auditory stimulus presented alone, by a tactile cue alone but not by visual stimulation. However, when auditory and visual stimulation was presented together, the response to the auditory cue was reduced. Note the higher response rates and spontaneous activity for neurons from hearing impaired animals (C–D) than in the animals with normal hearing (A–B).

Figure 4.

The proportion of unisensory and multisensory neurons found in hearing (A) and in adult hearing-impaired (B) core auditory cortex. White indicates unisensory neurons where Uni A = auditory; Uni V = visual; Uni T = tactile. Dark grey represents bimodal and trimodal multisensory neurons where AT = auditory-tactile; AV = auditory visual, and VAT = visual-auditory-tactile. Light grey shading indicates subthreshold multisensory neurons sAV = subthreshold auditory-visual; sAT = subthreshold auditory-tactile, sVAT = subthreshold visual-auditory tactile. Note that the proportion of multisensory neurons changed from approximately 34% of the hearing sample to 68% in the hearing-impaired.

Figure 5.

The activity of A1/AAF neurons in hearing ferrets (black bars) compared with that from adult hearing-impaired animals (gray bars). In **(A)**, neuronal spontaneous activity (spikes/second) was significantly (“*,” $p < 0.05$, t-test) lower in hearing animals than that measured from hearing impaired adults. In **(B)**, a similar trend was evident for responses to independent sensory stimulation (tactile alone, visual alone), where evoked activity was significantly (“*,” $p < 0.05$, t-test) lower in normal hearing ferrets than for the hearing-impaired animals. Error bars = standard error.

Figure 6.

Multisensory response change in multisensory A1/AAF neurons from (A) normal hearing ferrets and (B) from hearing impaired animals. In (A), responses of multisensory neurons from normal hearing animals to auditory stimulation (avg. 51 dB above hearing threshold) were, as a population, significantly depressed ($p < 0.001$, paired t-test) when combined with any of the non-auditory stimuli. The suppressive effect was consistent regardless of the modality of the non-auditory co-stimulation, as shown by the line of best fit (thick line) below the line (thin line) of unity for auditory-visual stimulus combinations (left), auditory-tactile combinations (center) or auditory-visual-tactile combinations (right). In contrast, the responses of the population of A1/AAF neurons from hearing impaired ferrets (B) to auditory stimulation (avg. 38 dB above hearing threshold) were not significantly affected by concurrent visual, tactile or combined visual-tactile stimulation. In each of these conditions, a line of best fit (thick line) through the data essentially overlaid the line of unity. Note the difference in axes-scales for hearing versus hearing-impaired data. Numbers at lower right of each plot indicate slope of the line of best fit and the correlation coefficient.

Figure 7.

Loss of multisensory integration in adult, hearing impaired animals. For multisensory core auditory neurons from normal hearing ferrets (black/dark bars) and hearing impaired animals (gray/light bars), these graphs compare the distribution of the magnitude of response change generated by combined stimulation when compared to the values elicited by the most effective separate modality stimulus. Percentages are calculated as a proportion of all sensory neurons sampled in each treatment group. (A) In normal hearing animals (black bars), the response change induced by combined-modality stimulation was strongly shifted in favor of negative or suppressed responses, with comparatively few responses occurring in

the positive direction. However, for multisensory neurons from hearing impaired animals (gray bars), combined-modality responses primarily generated little response change as the majority of measures occurred near zero. The average values for multisensory response change between the hearing and impaired animals was statistically significant (“*”, $p < 0.05$ t-test). **(B)** Responses to combined-modality stimulation that result in statistically significant ($p < 0.05$; paired t-test) increases (response enhancement) or decreases (response depression) in evoked activity are defined as examples of multisensory integration. Only those responses that met these statistical criteria are plotted (black=hearing; gray=impaired) as they represented significant levels of multisensory response integration. Because responses that show little change often do not meet the statistical threshold, most of the lower-magnitude response changes (e.g., between 20 and -20%) seen in (A) are not represented in (B). Integrated responses to combined-stimulation remained biased in the negative/suppressive direction for normal hearing animals (black bars), but not for the impaired ferrets. **(C)** Multisensory neurons from hearing (black/dark grey bar) or impaired (light grey bar) core auditory cortex showed significantly different (“*”, $p < 0.0001$; Chi-square) incidences of multisensory integration. Despite the fact that proportionally twice as many multisensory neurons were found in the hearing impaired animals (large gray bar), fewer of them showed significant multisensory response integration (labeled “Integrate”) than in the hearing controls. Conversely, proportionally more multisensory neurons from impaired animals than controls were not observed to integrate their multisensory inputs (labeled “Non-Int.”).

Table 1

Hearing and age statistics for the hearing impaired ferrets and for normal hearing adult ferrets. All test animals were adult males. The age of auditory critical period closure for ferret A1 is 80 days (Mrsic-Flogel et al., 2006) and the age for ferret sexual maturity is 150–180 days.

Animal ID	Hearing threshold	Days of age at ototoxic treatment	Days of age at recording
F09-021	45dB SPL	190	269
F09-022	55dB SPL	189	261
F09-023	35dB SPL	212	282
F09-034	55dB SPL	234	290
F09-035	55dB SPL	240	324
Average	49dB SPL	213	285
F10-014	15dB SPL	None - Control	315
F10-023	15dB SPL	None - Control	127
F10-024	15dB SPL	None - Control	145
F10-031	15dB SPL	None - Control	176
F10-032	15dB SPL	None - Control	449
Average	15dB SPL		242