Competition and convergence between auditory and cross-modal visual inputs to primary auditory cortical areas

Abbreviated title: Auditory and cross-modal inputs to auditory cortex

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Abstract

Sensory neocortex is capable of considerable plasticity after sensory deprivation or damage to input pathways, especially early in development. Although plasticity can often be restorative, sometimes novel, ectopic inputs invade the affected cortical area. Invading inputs from other sensory modalities may compromise the original function or even take over, imposing a new function and preventing recovery. Using ferrets whose retinal axons were rerouted into auditory thalamus at birth, we were able to examine the effect of varying the degree of ectopic, cross-modal input on reorganization of developing auditory cortex. In particular, we assayed whether the invading visual inputs and the existing auditory inputs competed for or shared postsynaptic targets, and whether the convergence of input modalities would induce multisensory processing. We demonstrate that although the cross-modal inputs create new visual neurons in auditory cortex, some auditory processing remains. The degree of loss of auditory input to MGN was directly related to the proportion of visual neurons in auditory cortex, suggesting that the visual and residual auditory inputs compete for cortical territory. Visual neurons were not segregated from auditory neurons, but shared target space even on individual target cells, substantially increasing the proportion of multisensory neurons. Thus spatial convergence of visual and auditory input modalities may be sufficient to expand multisensory representations. Together these findings argue that early, patterned visual activity does not drive segregation of visual and auditory afferents, and suggest that auditory function might be compromised by converging visual inputs. These results inform possible ways in which multisensory cortical areas may form during development and evolution. They also suggest that rehabilitative strategies designed to promote recovery of function after sensory deprivation or damage need to take into account that
sensory cortex may become substantially more multisensory after alteration of its input during development.

Introduction

It is well-documented that loss of sensory drive, whether as a result of sensory deprivation or brain damage, can result in cortical plasticity, especially early in development. The changes in circuitry that occur as a result of this reactive plasticity may help to restore function or may instead prevent restoration of normal function. Although cortical plasticity can involve either intra-modal or cross-modal plasticity, interference with normal function seems more likely to occur as a result of ectopic, cross-modal invasion of the deafferented structure. For example, loss of visual input can lead to auditory activation of visual cortex (Yaka et al. 2000) and loss of auditory input can lead to cross-modal activation of the understimulated auditory cortex by somatosensory or visual inputs (Allman et al. 2009; Bavelier and Neville 2002; Fine et al. 2005; Finney et al. 2001; Hunt et al. 2006; Lomber et al. 2010; Neville 1990; Neville et al. 1983; Nishimura et al. 2000; Sharma et al. 2007; Sterr et al. 2003). Cross-modal plasticity is known to interfere with the effectiveness of subsequently implanted cochlear prostheses in humans (Lee et al. 2001; see Sharma et al. 2009, for review). In order to promote restorative plasticity after sensory inputs are compromised and to minimize interference from cross-modal inputs it would be advantageous to understand how information from existing and ectopic inputs is coordinated, and in particular how cross-modal inputs affect the amount of territory devoted to the processing of the normal inputs.
Previous studies of sensory deafferentation or deprivation using animal models have examined cross-modal plasticity mainly from the perspective of a complete loss of normal input, such as bilateral enucleation or deafening by cochlear ablation. The disadvantage of using blind or deaf animals to study reactive plasticity in sensory cortex is that the original sensory modality can no longer be activated, preventing examination of how ectopic, cross-modal input affects recovery of the original function, whether through natural means or by implantation of a sensory prosthesis.

In this study we instead employ an approach that brings both normal and cross-modal information to sensory cortex from birth. We tested the hypothesis that cross-modal inputs compete with normal inputs for cortical space. One possible outcome of competition is that the smaller or less active input modality could be suppressed or taken over by the other.

Alternatively, segregation of neurons with different response modalities could occur, reducing cortical space available to each. A third possible outcome is that the cross-modal and the normal functions could coexist within the same cortical territory, expanding multisensory processing, or perhaps even converging onto single, multisensory neurons.

Using partial deafferentation of auditory thalamus in neonatal ferrets to examine how establishment of auditory cortical territory is affected by invasion of cross-modal, visual information, we find that, in addition to visual and auditory responses, multisensory responses are present at a rate much higher than that seen in normal auditory cortex. These three response types were not spatially segregated, suggesting that information carried by ectopic visual inputs is not sufficient to induce segregation. Our results demonstrate that primary auditory cortex can support both the original auditory and the novel visual function after recovery from damage to afferent pathways, and that multisensory function can be induced simply by experimental
convergence of two unisensory inputs. They provide insight into how multisensory cortex is created on developmental and evolutionary time scales. Additionally, our findings provide a more thorough understanding of the reorganization of an affected brain area after recovery from sensory damage or deprivation, and have important implications for rehabilitative strategies in patients with damage to sensory pathways.

Preliminary results from some of these experiments have been published previously in abstract form (Mao et al. 2007).

Materials and Methods

Partial deafferentation of auditory cortex (AC) and invasion of ectopic visual inputs can be produced in ferret kits if retinal axons are induced to invade auditory thalamus (MGN) (Sur et al. 1988) as a result of neonatal midbrain lesions. It has been shown that the cross-modal auditory cortex (XMAC) in similarly-manipulated animals contains functional visual neurons (Roe et al. 1992; Sur et al. 1988; von Melchner et al. 2000). Auditory and multisensory responses were not reported in these previous studies, perhaps because the aim was a complete deafferentation of MGN.

Animals

Data were obtained from 25 pigmented ferrets (Mustela putorius furo) aged 4 months or more (ferrets reach full brain and body size at 16 weeks (Fox and Bell 1998)). Timed pregnant ferrets were obtained from Marshall Farms (North Rose, NY) two weeks prior to parturition. Nursing dams and kits were fed a high fat diet and kept on a 14h/10h light/dark cycle. Kits were weaned
at 6-8 weeks of age. Normal ferrets were obtained either from Marshall Farms as adults or bred
in our colony. Non-lactating ferrets were fed Marshall Farms ferret diet and kept on a 12/12
light/dark cycle. Both male and female ferrets were included in the study. All protocols were
approved by the Institutional Animal Care and Use Committee (IACUC) at Georgia State
University and met or exceeded standards of care established by the USDA and the Society for
Neuroscience.

**Neonatal surgery**

Surgical procedures were similar to those described previously (Pallas et al. 1999). Ferret kits
were manipulated within 24 hr after birth. They were anesthetized by isoflurane (1-4%). All
surgeries were performed under sterile conditions. After a kit was anesthetized, the skull over the
midbrain was exposed and removed with a scalpel. The left superior colliculus and one or both
inferior colliculi were then lesioned to varying extents with a heat cautery, and the brachium of
the left inferior colliculus was severed with a scalpel blade. The incision was closed using either
6-0 prolene or surgical adhesive (VetBond, 3M, St. Paul, MN). After surgery, the kits were
given subcutaneous fluids and a respiratory stimulant (doxapram, 2 mg/kg, SQ) and warmed
under an incandescent lamp. Kits were observed carefully until they recovered from anesthesia,
and were returned to their dam after they became ambulatory. Analgesics (buprenorphine 0.05–
0.1 mg/kg bid) were given as needed to prevent postoperative pain.

**Preparation for Electrophysiology**

Electrophysiology experiments were done once ferrets reached adult size (>4 months of age).
Before induction of anesthesia, atropine (0.4 mg/kg SQ,) and doxapram (2 mg/kg, SQ) were
given to counteract bradycardia and to reduce mucosal secretions. Anesthesia was induced by ketamine (40mg/kg, IM) and diazepam (2mg/kg, IM) or by ketamine (40mg/kg, IM) and medetomidine (0.08 mg/kg IM). Dexamethasone (1mg/kg, IM) was given every 24 hours to prevent brain swelling. Animals were intubated and the cephalic or femoral vein was cannulated. Anesthesia was maintained with an IV solution of medetomidine (0.022 mg/kg/hr) and ketamine (5 mg/kg/hr) in lactated Ringer’s with 5% dextrose (Bizley and King 2008; Bizley et al. 2005). Atropine (0.06 mg/kg/hr, SQ) was given as necessary to counteract the bradycardia caused by medetomidine. Animals were artificially respired using a small animal ventilator (SAR 830/P ventilator, CWE Inc, Ardmore, PA). Vital signs including EKG, respiration rate, muscle tone, withdrawal reflexes, end-tidal CO$_2$, and SpO$_2$ were monitored during the surgery and recordings to ensure maintenance of adequate anesthesia. Body temperature was maintained at 38°C with a heating pad. Pupils were dilated with atropine ophthalmic drops. Eyes were kept moist with commercial artificial tears solution and protected with custom plano contact lenses (Conforma Inc, Norfolk, VA). The head was stabilized in a stereotaxic device. After the scalp overlying auditory cortex was incised and the muscle was retracted from the skull, two burr holes (at coordinates A5.5 ±L1.5) were drilled for optic chiasm recording/stimulation electrodes. Two tungsten rods with Teflon insulation (0.008 bare, 0.011 coated, A-M Systems, Inc., Carlsborg, WA) connected to a preamplifier were lowered (8~10 mm) while recording responses to strobe light stimulation until a depth yielding strong visual responses was reached. These tungsten rods were then cemented to the skull and connected to a stimulus isolation unit (BAK Electronics, Inc, Mount Airy, MD). A 0.8~1.0 cm diameter craniotomy was drilled over the auditory cortex and the suprasylvian and pseudosylvian sulci were exposed.
**Recording sites:** The ferret AC is located on the middle ectosylvian gyrus, bounded above by the anterior and posterior arms of the suprasylvian sulcus (sss) and below by the pseudosylvian sulcus (pss) (Kelly et al. 1986). In this study, recording penetrations were targeted to primary auditory cortex (A1) and anterior auditory field (AAF) (Bizley et al. 2005) and were unlikely to impinge on multisensory regions of the sulci surrounding AC for several reasons. First, we avoided the regions close to the sulci. In addition, our recording depths were generally very superficial given that we recorded the first unit in each electrode pass then moved on to the next location in the map. Further, the electrode angle combined with the shape of AC as an inverted parabola is such that increasing recording depth would move away from and not toward the banks of the sulci. These factors taken together argue that it is very unlikely that our recording sites were in the suprasylvian territory, although we cannot exclude the possibility. We cannot exclude the possibility that a few of our recording sites were located in the posterior ectosylvian gyrus, where higher-level auditory fields have been described (Bizley et al. 2005).

The dura was removed and AC was covered by either sterile saline or 2% Agar (Fisher Scientific, Fair Lawn, NJ) in sterile saline to protect its surface from desiccation. The right side of the skull was cleaned of tissue and a metal bracket was cemented on the skull to hold it in position. The right ear bar was then released to allow access to the ear for auditory stimulation.

**Extracellular recording**

The cortical surface was photographed with a digital camera in order to record the location of each recording electrode penetration. A glass-coated tungsten microelectrode (1-2 MΩ, FHC, Bowdoin, ME) was used to investigate neuronal activity in AC in response to auditory and visual stimuli. Penetration locations were chosen to sample randomly from as many sites within AC as
possible while avoiding sulci and blood vessels. The electrode was lowered in 5 µm steps up to 2000 µm under the pial surface using a hydraulic microdrive (Kopf Instruments, Tujunga, CA).

Once the first unit in each electrode pass was isolated and characterized, another recording location was selected. Most units were isolated within 800 µm of the pial surface.

Sensory stimuli: For each penetration, bars of light from a pantoscope and white noise from a loudspeaker were used to search for responsive neurons. The loudspeaker was placed at a 45° angle between the right side and the front of the animal at a distance of approximately 10 cm.

Auditory searching stimuli were white noise bursts (5 ms ramp, 40-100 ms duration) with a sound intensity of 60-80 dB SPL, as measured by a sound level meter (model 407764, Extech Instruments, Waltham, MA). After a responsive neuron was found, computer-generated auditory (noise or tones) and visual stimuli (moving or flashing bars of light) were used for testing responses. The speaker was replaced with a calibrated earphone. The earphone was placed in the pinna at the entrance to the ear canal and used to generate noise bursts or pure tone sounds in closed field. (ER-2 insert earphone, Etymotic Research, IL). The earphone was calibrated with a microphone (ER-7C probe microphone system, Etymotic Research, IL) via Sigcal software (Tucker-Davis Technologies, Alachua, FL). The normalized file generated by Sigcal was used to correct any non-linearities in the earphone output when sound was given. For assessing the responses of single units isolated in each penetration, auditory stimuli were generated by TDT System II hard- and software (Tucker-Davis Technologies, Alachua, FL) and visual stimuli were synthesized and delivered by a VSG card (Cambridge Research Systems Ltd, Kent, England).

Light stimuli included moving bars or gratings at eight orientations moving in either direction, presented on a computer screen ~40 cm distance from the eyes. Bipolar electrical stimulation of
the optic chiasm was applied (single pulses at 0.5-1 mA, 60 µs duration) in addition to light stimuli.

Electrophysiological data analysis

Neural responses were amplified (5000 to 10000 times, BAK Electronics, Inc, Mount Airy, MD), filtered (500 Hz to 5 kHz), and monitored on a digital oscilloscope (Hameg Instruments, Mainhausen, Germany). Responses to 10-15 stimulus presentations were gathered from each recording site and digitized at 25 kHz. The evoked responses were averaged and normalized to a sample of spontaneous activity recorded 50 ms before each trial. The recording continued for 1-2 days, after which the animal was deeply anesthetized with sodium pentobarbital (65 mg/kg) for humane euthanasia and harvesting of brain tissue for histological examination.

For each electrophysiological data point, Brainware software (Tucker-Davis Technologies Inc., Alachua, FL) was used off-line to isolate extracellularly recorded spikes derived from single neurons by their waveform. Artifact rejection was set in Brainware to extract biphasic action potential candidates with both peaks exceeding background noise level. Spikes with similar shape and duration were shown as clusters in the data-sorting window. Post-stimulus time histograms (PSTHs) of the selected single units were generated using the same software package. The mean and standard error of the number of spikes to each stimulus presentation were calculated after subtracting spontaneous activity. Response latencies were determined by the time between stimulus presentation and the time of the first bin in the PSTH that reached at least 20% above background firing rate. Multisensory units were defined either as neurons that responded both to visual and auditory stimuli or as neurons that only responded to one modality but could be significantly modulated by stimulation with the other modality (Stein et al. 1993).
Statistically significant differences were determined by comparing the number of spikes per sweep (obtained from the PSTHs) as a response to different stimulus modalities using Student’s t-test (p<0.05). The proportion of response types was compared across groups.

For calculating the spatial distribution of response types, the area of each AC was normalized to a standard circle with a radius of 1. The locations of recorded units were reconstructed on this normalized AC. In order to analyze the distribution of different neuron types in AC, we divided AC into four quadrants numbered 1 to 4 as seen in Figure 1. The quadrants were not intended to correspond to particular auditory cortical areas, although quadrants 2 and 3 overlap more with the anterior auditory field (AAF) and quadrants 1 and 4 overlap more with primary auditory cortex (A1). Two lines were drawn along the anterior and posterior arms of the suprasylvian sulcus to form angle A. The third line was drawn just above the tip of the pseudosylvian sulcus and perpendicular to the dividing line of angle A. The center of the internally tangent circle (point 0) was defined as the intersection of the dividing lines of angle A and angle B.

Because the shape of the AC in each individual is unique, and the location of recording sites differed somewhat across animals, we examined whether pooling data from different animals into one polar plot would bias the data. We performed a heterogeneity Chi-square analysis to test the homogeneity of data from each group. Heterogeneity Chi-square is a statistical test based on the premise that if the samples are homogeneous, then the value of $\chi^2_{\text{sum}}$ should be close to the value of $\chi^2_{\text{pooled}}$. Therefore, the heterogeneity Chi-square value is designated ($\chi^2_{\text{het}} = \chi^2_{\text{sum}} - \chi^2_{\text{pooled}}$). The null hypothesis should be rejected if there is a large $\chi^2_{\text{het}}$ (for details, see Sheskin
2004). If the value of the sum of Chi-squares from each sample is not significantly different from
the value of the pooled Chi-squares (p > 0.05), \( \chi^2_{het} \) will be small and the data can be grouped.

Applied to our data, the test showed that the electrode penetrations in the four quadrants of
normal AC were homogenously distributed (p > 0.05). The same was true for small lesion and
large lesion groups (p > 0.05). Therefore, data from all ACs in each group were pooled into one
polar plot. A Chi-square analysis was then applied to determine whether recorded neurons were
randomly distributed across quadrants independent of their response type. In cases where the
distribution was not random (p < 0.05), an Analysis of Residuals (R value) was calculated to show
which quadrant(s) contained the unexpected distribution.

To examine whether neurons with similar responses were clustered, we calculated the
average distance between recording sites by translating X and Y values obtained from
normalizing AC to polar coordinates on the standard circle using Microsoft Access database
software. The distance between each pair of single units was calculated and exported to a
spreadsheet. The average distances from each single unit to other auditory, visual or
multisensory units were calculated. Then we compared the mean of average distance between
pairs across groups.

Electrophysiological data were statistically analyzed using Sigmasstat software (Systat
Software Inc, Chicago, IL) and plotted with Sigmaplot (Systat Software Inc, Chicago, IL). A one
way ANOVA for multiple groups was used. A Tukey post hoc test was used for groups that had
uneven numbers, and a Fish LSD post hoc test was used for groups that had even numbers of
members. A Mann-Whitney U test for non-normally distributed data was used for two group
comparisons. Means are given with standard errors of the mean (± SEM) throughout.
Assessment of lesion size

MRI Scanning: Magnetic Resonance Imaging (MRI) was performed in some lesioned ferrets to obtain an assessment of the midbrain lesions prior to electrophysiological recording. Atropine (0.4 mg/kg SQ) and doxapram (2 mg/kg SQ) were given 5 min prior to sedation. Then, medetomidine (1 mg/kg, IM) and diazepam (2 mg/kg, IM) were given to sedate the animal. Animals were put into an MRI cradle with a heating pad underneath to maintain body temperature. End-tidal CO₂, SpO₂, pulse rate, respiration rate, and body temperature were monitored during the entire process. MRI scanning of the midbrain was normally finished within 30 min. Animals were taken out of the cradle and given atipamezole (0.5 mg/kg, IM) to reverse the effects of the medetomidine. Animals were then continuously monitored over the next 1 to 2 hours before being returned to the colony to ensure that they were completely recovered from the drugs.

Histology: After electrophysiology, animals were deeply anesthetized with sodium pentobarbital (65 mg/kg) for euthanization and perfusion with phosphate-buffered saline (PBS) followed by 2%-4% paraformaldehyde in 0.1M PB. Brains were extracted, postfixed in 4% paraformaldehyde in 0.1M phosphate buffer (PB) for 24 hr, and stored in 30% sucrose in 0.1M PB at 4º C. After the tissue was infiltrated by the sucrose solution, it was sectioned frozen at 50μm in the coronal plane for reconstruction of lesions. A series of sections at 200 μm intervals was stained for Nissl substance using cresyl echt violet.

Analysis: The size of the residual central nucleus of the inferior colliculus (ICc) and the superficial layers of the superior colliculus (sSC) in each animal’s midbrain was measured from Nissl stained sections with a Zeiss microscope using Zeiss Axon Vision 3.1 software (Carl Zeiss MicroImaging, Inc., Thornwood, NY). The borders of ICc and sSC (areas indicted by the dashed
line in Figure 2A, B and dark areas in Figure 2C) were very clear on our Nissl stained sections. The volume of sSC and ICc was calculated as the sum of each measured areas multiplied by 200 $\mu$m. Proportions of residual midbrain area and volume in the lesioned animals were calculated by comparison with an average midbrain volume derived from five normal animals. Lesioned animals were sorted into small and large lesion groups as determined by these measurements (Figure 2).

Figure 2 here

Results

Twenty-five ferrets in total were used in this study. Ten were entered in the normal group and 15 received neonatal lesions leading to cross-modal plasticity. Below we characterize and compare the response properties of the 401 AC neurons recorded in the normal group and the 573 AC neurons recorded in the lesioned group.

Normal AC contains primarily auditory responses plus rare multisensory responses

Normal animals were used in the experiments as a negative control for the effects of the midbrain lesion. Although primary sensory cortices are traditionally defined as brain areas that respond only to a single sensory modality, recent research has challenged this view by reporting the existence of multisensory neurons and neurons responding to other modalities in primary sensory cortices. Bizley and colleagues have reported that primary auditory cortex in ferrets does contain some auditory/visual bisensory and some visual neurons (Bizley et al. 2007). In order to investigate whether and to what extent primary auditory cortices (A1 and AAF) in normal ferrets
can respond to visual stimuli under our experimental conditions and methods of analysis, we characterized the response modality of 401 single neurons in AC of 10 normal animals using in vivo extracellular recording. We defined auditory neurons and visual neurons as those that responded to only one modality. Multisensory neurons were defined as those that either responded to both modalities or responded to one modality but were significantly modulated by stimulation from the other modality (criterion of p<0.05, t-test on number of spikes to single vs. bimodal stimuli, 10 trials or in some cases 15 trials, data obtained from PSTHs) (see Meredith and Stein 1986). We found that 11% of the 401 neurons recorded in AC of normal ferrets were multisensory. These multisensory neurons responded both to sound stimuli and to electrical stimulation of the optic chiasm (n=45, Fig. 3A), but not to stimulation by light. No visual-only neurons were found in our sample of normal animals.

XMAC contains visual, auditory, and multisensory response types

We next tested whether auditory responses remain in AC of lesioned animals and whether the ectopic visual inputs to MGN were associated with an increased proportion of multisensory or visual-only units. We predicted that XMAC’s residual inputs from auditory areas would preserve auditory responsiveness, despite earlier reports to the contrary (Roe et al. 1992; Sur et al. 1988). Callosal connections between XMAC and AC in the unlesioned hemisphere exist (Pallas et al. 1999), and the inferior colliculi are incompletely lesioned in many cases. These inputs could confer auditory responses on XMAC. In support of this prediction, our data showed a high proportion of auditory neurons in XMAC despite the presence of ectopic visual responses. Multisensory neurons were also found. In all of the lesioned animals considered together, the relative proportion of auditory-only neurons was 56%, the proportion of multisensory neurons
was 32%, and the proportion of visual-only neurons (optic chiasm and/or light driven) was 12% (Fig. 3B). Post-stimulus histograms are shown for each response type in Fig. 3C-F. The existence of auditory neurons in XMAC reveals that the neonatal midbrain lesions and ectopic visual inputs do not eliminate or suppress the auditory function of AC. The presence of a higher than normal proportion of multisensory neurons in XMAC suggests that auditory and visual inputs are more likely to converge in XMAC than in normal AC.

Figure 3 here

Continuous and categorical differences in response type occurred by altering the extent of midbrain sparing

The above finding that auditory and visual responses can be made to coexist in XMAC allowed us to address the relationship between the two response types in more detail, and in particular to examine how the induction of visually-responsive areas in auditory cortex would affect normal auditory processing. In patients with a sensory deficit or damage that deafferents a brain area, invasion of cross-modal inputs often occurs to varying extents, and at some point may become maladaptive. We wished to determine whether progressively increasing the extent of visual invasion of XMAC would result in competition (intermodal suppression) or cooperation (multimodal convergence) between modalities. Given previous reports that auditory responses are absent in XMAC (Roe et al., 1992), we wanted to test whether increasing levels of visual input activity would suppress or eliminate auditory responses. Such a finding could explain why we observed auditory responses in our data set whereas none were found in the Roe et al. (1992) study in which very large lesions were made.
In order to investigate the effect that increasing degrees of invasion of ectopic visual inputs would have on auditory responsiveness in AC, we measured the midbrain lesion size in each ferret using histological techniques, and compared this measure with the relative proportions of each response type in a systematic fashion. We quantified midbrain size of the lesioned animals by comparing the volume of the left and right central nucleus of the inferior colliculus (ICc) and the left and right superficial superior colliculus (sSC) in each lesioned animal to that averaged across five normal animals used as a standard of comparison (cf. Figure 2, Table 1).

Table 1 here

Next, it was necessary to demonstrate that increasing the lesion size would affect the relative proportions of auditory, visual, and multisensory neurons in XMAC. We found that midbrain lesion size was correlated with the proportion of auditory and visual response types in an interesting way. In general, overall lesion size was correlated with the proportion of visual units and inversely correlated with the proportion of auditory units (Fig. 4A, D). There was a tighter relationship between spared sSC size and visual responsiveness ($r = -0.7$, $p = 0.006$) than between spared ICc size and visual responsiveness ($r = -0.48$, $p = 0.085$; compare Figs. 4B and C). These results suggest that establishment of visual neurons in XMAC relies more on damage to visual midbrain than to auditory midbrain. For auditory responsiveness, the correlations with total midbrain size, sSC size, and ICc size were similar to each other (Figs. 4D-F). These data show that residual sSC volume predicts the relative proportions of visual and auditory neurons, whereas ICc volume is predictive only of the proportion of auditory neurons, demonstrating that the amount of retinal target area lost (SC lesion) is crucial for determination of neuron types in
XMAC when both ICc and sSC are lesioned. It was also notable that even complete ablation of left IC did not eliminate auditory responses in XMAC (Table 1). We did not find any correlation between the proportion of multisensory units and the amount of spared auditory or visual midbrain.

The relationship between lesion size and proportion of auditory units (Fig. 4D) appeared roughly linear, with increasing lesion size correlating with a decreasing proportion of auditory responses. In terms of visual responsiveness, however, as may be predicted by examination of Figure 4A and 4B, there was evidence of an exponentially decreasing relationship (Fig 4A, r=0.8, p=0.0007; Fig 4B, r=0.88, p<0.0001, exponential fit) or perhaps a categorical response to lesion size rather than a progressive, linear response.

XMAC contained light-responsive neurons (that is, neurons that responded to light as well as to optic chiasm stimulation) only in animals in which most of the left midbrain was ablated (less than 10% residual left midbrain). In animals with more than 10% residual left midbrain there were no light-responsive neurons. We used this categorical distinction to divide the cases into a large lesion or small lesion group, respectively, and conducted further analyses according to these categories. We performed statistical analysis to examine whether small vs. large lesion groups have significantly different residual midbrain sizes. We found that the spared left midbrain size of animals in both the large (5.4± 1.76%, n=5) and small lesion groups (43.7± 5.34%, n=10) was significantly smaller than that of the normal group (ANOVA, p<0.001, Fig. 5). The spared midbrain size in the large lesion group was also significantly reduced compared
to that in the small lesion group (Tukey post hoc test, p<0.001), allowing us to consider these
groups along with the unlesioned group as distinct categories for statistical analyses.

**Figure 5 here**

*Responsiveness to light requires minimal sparing of visual midbrain*

In order to determine the relationship between lesion group membership and distribution of
response types, we calculated the relative proportions of auditory, visual, and multisensory
responders in each group. This analysis allows us to compare the proportion of neuronal
responses among groups, including the proportion of multisensory neurons that has not been
shown in the correlation analysis.

Animals in the small lesion group had few visual neurons in XMAC (1.8%± 0.96%, n=8 of
414 neurons from 10 animals) and those neurons responded to electrical stimulation of the optic
chiasm (OX) but not to light. The low proportion likely results from the minimal redirection of
retinal axons to MGN in small-lesion cases (Angelucci et al. 1998). In contrast, 33.8 ± 7.92% of
recorded neurons in the large lesion group were visual neurons (n=61of 159 neurons from 5
animals, **Fig. 6A**). Of the 61 visual neurons, 48 of them (78.7%) responded to light in addition
to optic chiasm stimulation. This is a significant increase (Mann-Whitney U-test, p=0.003) and
represents a categorical difference between the large and small lesion groups. These results
indicate that considerable visual information was reaching AC in the large lesion animals.

*Auditory neurons become visual rather than converting to multisensory neurons*
Although auditory neurons were found in all groups, the proportion of auditory neurons to total recorded neurons in both groups of lesioned animals varied with lesion size (large lesion group: 43.8± 6.47%, n=65 of 159; small lesion group: 60.0± 3.22%, n=256 of 414, normal group: 88.6± 1.65%, n=356 of 401, ANOVA, p<0.001, Fig. 6B). Furthermore, the proportion of auditory neurons in large lesion groups was less than that in the small lesion group (p=0.003, Tukey post-hoc). These results suggest that increasing lesion size resulted in an increase in visual neurons largely at the expense of auditory neurons, rather than a conversion of auditory neurons into multisensory neurons.

**Multisensory neurons were found in all groups**

We found that neurons in AC of all three groups responded to both auditory and optic chiasm stimulation (multisensory neurons in the large-lesion group: mean 22.4± 5.60%, n=33 of 159; in the small-lesion group: mean 38.3± 3.51%, n=150 of 414, in the normal group: mean 11.5± 1.65%, n=45 of 401, ANOVA, p<0.001, Fig. 6C), but interestingly that the proportion of multisensory neurons in the small-lesion group was significantly higher than the proportion of multisensory neurons in the normal group (Tukey post hoc test, p<0.001) and in the large lesion group (Tukey post hoc test, p<0.05). Although the large-lesion group contained more multisensory neurons than did the normal group, statistical analysis indicated that there was no significant difference (large vs. normal, p=0.11). This probably results from the large proportion of purely visual neurons in the large lesion group, and suggests that the response of XMAC to minimal invasion of visual activity (making multisensory neurons) is different from its response to substantial visual influence (making purely visual neurons). This result has interesting implications for rehabilitation strategies for patients with sensory loss or brain damage that cause
cross-modal redirection of afferent inputs. Selective stimulation of the original modality would be expected to produce very different results depending on the amount of redirection.

**Figure 6 here**

**Auditory and multisensory neurons have distinct spatial distributions in normal animals**
To determine whether neuronal responses to different modalities are preferentially located in one or multiple regions of AC, we calculated the distribution of recorded neurons in the normal AC across the four quadrants (cf. Fig. 2). **Figure 7** shows examples of the penetration locations and neuronal response types in recordings of normal AC from 8 of the 10 normal animals. We found that auditory neurons were distributed randomly across the entire AC, but most multisensory neurons were located laterally. Using statistical analysis, we found that auditory neurons were evenly distributed (Chi-square, p>0.05). However, the distribution of multisensory neurons was skewed to one of the four quadrants (Chi-square, p<0.001, analysis of residuals, R=6.93> R.<001=3.29) (**Figure 8**). The polar plot (Fig. 8A) shows that the population of multisensory neurons was preferentially located in the lateroposterior (quadrant 4; see Fig. 2), in normal animals. The observed incidence of multisensory neurons in quadrant 4 was significantly higher than the expected value of 25% (Fig. 8B). These data show that auditory responses in normal animals are distributed evenly across the AC, whereas multisensory neurons are preferentially located lateroposteriorly.

**Figures 7 and 8 here**
The spatial distribution of auditory, visual and multisensory neurons in lesioned animals was different from normal

Spatial distribution of neuronal response types in small-lesion cases

We next investigated whether neurons with auditory and visual responses in AC of small lesion animals would be segregated as seen for multisensory neurons in normal auditory cortex. Such clustering would likely facilitate efficient processing of visual information separately from auditory information after the midbrain injury. Eight examples of raw data are presented in Figure 9 to indicate recording locations and neuronal response types in each small lesion animal. Pooled data from all 10 small lesion animals showed that auditory neurons in small lesion AC were randomly distributed across the four quadrants (Chi-square, p>0.05, Fig. 10). Multisensory neurons could also be found in any of the quadrants in XMAC of small-lesion animals, but were more likely to be found in Q4. The number of multisensory neurons in the anteromedial quadrant Q1 was below the expected value for a random distribution (Chi-square, p<0.01, analysis of residuals, R=2.79>R_{0.01}=2.58, Fig. 10B), and the number in the lateroposterior quadrant Q4 was above the expected value (Chi-square, p<0.001, analysis of residuals, R=3.61>R_{0.001}=3.29, Fig. 10B). In addition to auditory and multisensory neurons, we recorded some purely visual neurons (8 out of 414 neurons), that were located exclusively in lateral AC (dark triangles in Fig. 10). These results suggest that ectopic visual inputs can invade the entire AC, even though they are concentrated in the lateroposterior quadrant. This is interesting given the result that quadrant Q4 already contains multisensory neurons in normal animals (Fig. 8). Although the multisensory neurons were preferentially located in the lateroposterior quadrant of both normal and small lesion animals, the extent of clustering in small lesion animals was reduced compared to that in normal animals. These findings imply that the random distribution
of ectopic visual inputs to XMAC may weaken the tendency of visually-responsive neurons in normal AC to cluster.

In addition to the analysis of distribution of neuronal responses in the four quadrants, we measured the distance between each single unit and its neighbors in order to further test the segregation hypothesis. We found that the average distance between pairs of sound-responsive neurons was significantly shorter than the average distance between auditory and multisensory neurons (ANOVA, p<0.05, A-A vs. A-M, Fisher LSD method, Fig. 10C). This finding suggests that auditory neurons are more likely to cluster with each other than with multisensory neurons. This result may be attributable to the smaller number of multisensory responses compared to auditory responses in XMAC of small lesion animals. We were prevented from including visual responses in the analysis because some animals had only one visual neuron in the entire AC.

Figures 9 and 10 here

Spatial distribution of neuronal response types in large lesion cases
Because some response type clustering was seen in AC of small lesion cases, we wondered if the more extensive visual inputs resulting from larger lesions would either promote or reduce clustering of neuronal response types in XMAC. Note that it is difficult to generate animals with large midbrain lesions, thus there is a smaller number (n=5) of large lesion cases compared to small lesion cases (Fig. 11). We found that auditory, visual and multisensory neurons were evenly distributed across the four quadrants of AC in large lesion cases (Chi-square, p>0.05, Fig. 12). This was different from normal and small lesion cases, in which the multisensory and visual neurons were preferentially located in the lateroposterior quadrant Q4. Although there was
variation in the sample, there was no significant degree of segregation of neuronal response types in XMAC of the large lesion group. These results suggest that the ectopic visual inputs introduced by the large neonatal midbrain lesions projected evenly across the entire AC rather than being more strictly segregated as we expected.

As with the small lesion group, we measured the distance between pairs of auditory, visual and multisensory neurons in the large lesion group to test the hypothesis that neurons with similar response properties would be clustered together. We compared the average distance between pairs of auditory neurons to the average distance between auditory-visual pairs and auditory- multisensory pairs of neurons. We did not find any significant tendency to cluster (ANOVA, p>0.05, A-A vs. A-M or A-V, Fisher LSD method, Fig.12C), nor did we find any significant clustering when we compared average distance between pairs of visual neurons to the average distance between visual-auditory and visual- multisensory pairs (ANOVA, p>0.05, V-V vs. V-A or V-M, Fisher LSD method, Fig.12C). We did find that the average distance between pairs of multisensory neurons was significantly shorter than the average distance between multisensory -visual pairs (ANOVA, p<0.05, M-M vs. M-V, Fisher LSD method, Fig. 12C), but found no significant difference between M-M and A-M pairs. These results suggest that multisensory neurons are closer to each other than to visual neurons. Overall, these results are in agreement with our polar plot data and further suggest that auditory and visual inputs to AC of large lesion animals do not segregate.

Figures 11 and 12 here
The latency of visually-responsive neurons to optic chiasm stimulation differed between normal and lesioned animals. Previous research demonstrated that ectopic visual inputs to XMAC come from the retino-MGN-AC projection (Pallas et al. 1990b; Sur et al. 1988), whereas visual inputs to normal AC come from corticocortical projections (Bizley et al. 2007). We found clustering of multisensory neurons lateroposteriorly in normal AC, whereas in AC of large lesion animals, multisensory neurons were randomly distributed. To investigate whether visual inputs to multisensory and visual neurons in lesioned animals came from expanded corticocortical projections or from retino-MGN-AC afferents, we compared the latency of responses to optic chiasm stimulation. The latency to optic chiasm stimulation of the multisensory neurons recorded in normal animals (n=40) was 13.4 ± 1.75 ms whereas the latency to optic chiasm stimulation of multisensory and visual neurons from lesioned animals was 8.76 ±1.03 ms (n=86) in the small lesion group and 5.72 ± 0.65 ms (n=65) in large lesion group, which was significantly shorter (ANOVA, small vs. normal p=0.015, large vs. normal, p<0.001, Fig. 13). No significant difference was found between small and large lesion groups. The comparison between latency of response to optic chiasm in normal visual cortex and Xmodal auditory cortex was reported in a previous study in which the authors showed that visual neurons in Xmodal AC have longer latencies than visual neurons in visual cortex (Roe et al. 1992). Taken together, these results provide further evidence to support the contention that normal AC receives its visual inputs indirectly, perhaps from other cortical areas, but not directly from thalamus, whereas XMAC receives visual inputs more directly, probably from the retina to MGN to AC pathway.

Figure 13 here
Discussion

Early lesions to sensory structures in one sensory modality can result in profound reorganization across multiple sensory pathways, due to the many interconnections between structures (Karlen et al. 2006; Kingsbury et al. 2000; Kingsbury et al. 2002; Pallas et al. 1990a; Pallas and Sur 1993). The fact that such reorganization can result in cross-modal connections has important clinical implications for recovery from perinatal brain damage because the different modalities could either cooperate or compete with each other. We found that auditory neurons and multisensory neurons coexist with visual neurons in XMAC after recovery from neonatal midbrain damage. The existence of multisensory neurons indicates that the two modalities can converge and cooperate to activate individual target neurons, rather than competitive suppression of one input by the other. The proportions of auditory and visual neurons were directly related to the amount of residual midbrain tissue. Rather than being segregated from visual neurons, auditory neurons were evenly distributed across XMAC of lesioned animals, and an increase in the number and a broadening of the distribution of multisensory neurons was observed. These findings are reminiscent of phenomena such as acquired auditory-tactile synesthesia, which was reported in a patient following recovery from a thalamic infarct. In this patient, sound induced BOLD responses in somatosensory cortices (Beauchamp and Ro 2008; Ro et al. 2007). Collectively, these results suggest that both cooperation and competition between the two input modalities are involved in the reorganization of sensory areas after damage to sensory inputs.

Auditory function is retained in core auditory cortical regions despite visual inputs
Previous studies using neonatal midbrain lesions in ferret kits focused on visual responses in XMAC (Roe et al. 1990, 1992; Sur et al. 1988; von Melchner et al. 2000). Those studies, which used very large midbrain lesions, reported that there was no residual auditory function in cross-modal AC. We used lesions of varying size, and find that considerable auditory function is retained despite the visual input, especially in animals with smaller lesions. This fortuitous finding allowed us to examine the effect of different degrees of cross-modal input on development and plasticity of auditory cortex in response to deafferentation.

Our finding that a large proportion of the neurons in XMAC retain auditory responsiveness supports the hypothesis that residual auditory afferents can compensate even for complete loss of the ipsilesional inferior colliculus, but raise the question of where the auditory information derives from. The most likely sources of auditory input to XMAC are the ipsilateral MGN (as a conduit of input from spared contralateral ICc (Angelucci et al. 1998; Moore et al. 1998) and the contralateral auditory cortex (via the corpus callosum (Pallas et al. 1999)) (Fig. 14). Whether the auditory neurons in XMAC function as they would in normal animals is an important question and is the subject of a current study. Preliminary results suggest that function is somewhat compromised (Mao and Pallas 2009).

Figure 14 here

**Competition between visual and auditory inputs may determine response type**

Our results show that with decreasing size of IC and SC, the percentage of recorded neurons responding to sound went down, and the percentage of visual neurons went up. These results are consistent with competition as an explanatory mechanism (Crair et al. 1997; Hubel and Wiesel
1962; Stryker 1982; Stryker and Harris 1986). During recovery, when the normal sensory drive may be maximally compromised, activity-dependent processes may allow invading, cross-modal inputs to out-compete preserved inputs from the normal pathway. These findings suggest that optimizing rehabilitation of patients suffering from sensory dysfunction or brain damage will require not only increasing the activity of the original inputs but also decreasing activity in the ectopic inputs. For example, rearrangement of somatosensory circuits in early blind humans can degrade somatosensory representations (see Sathian and Stilla 2010 for review; Sterr et al. 2003) and cross-modal changes in deaf patients can interfere with the success of cochlear implants (Lee et al. 2001).

Our findings may also be of relevance to studies on recovery from partial deafness following cochlear damage. Fallon et al (2009), using neonatally deafened cats, found that large portions of A1 were non-responsive to sound activation of cochlear implants. These non-responsive regions may actually be visually responsive, perhaps interfering with the efficacy of cochlear implants due to loss of territory for sound processing. Similar loss of auditory cortical territory for sound processing may result from damage to IC due to disease or injury (Bognar et al. 1994; Hoistad and Hain 2003; Kimiskidis et al. 2004; Lee et al. 2009; Masuda et al. 2000; Meyer et al. 1996; Musiek et al. 2004). Understanding how to manipulate competition between sensory modalities converging on a cortical territory would be helpful in designing clinical therapies.

Unisensory auditory or visual neurons are intermingled within cross-modal AC

In contrast to research on cortical plasticity within one modality, this study addressed how ectopic, cross-modal inputs that invade a deafferented cortex affect normal function. We examined the possibility that both modalities could function independently through the
segregation of their representations in XMAC. Neurons with similar response properties tend to be clustered together in unisensory sensory cortex (Hubel and Wiesel 1962; 1963; see Mountcastle 1997 for review) and in multisensory cortex (Dahl et al. 2009). Activity-dependent sorting of inputs can drive spatial segregation of different response types (Miller et al. 1989; Reh and Constantine-Paton 1985). As in other mammals, primary auditory cortex in ferrets maps sound frequency in one-dimension (Kelly et al. 1986; Phillips et al. 1988), whereas primary visual cortex maps visual space in two-dimensions (Law et al. 1988). If this remains the case in XMAC, it seems unlikely that visual and auditory neurons would be simultaneously active. It has been suggested that evolutionary pressure causes neurons with similar response properties to group together in order to reduce axon length and connection distance (Chklovskii and Koulakov 2004; Chklovskii et al. 2002; Kaas 2006; Ringo 1991). Such a tendency would also reduce the difficulty of wiring developing circuits appropriately. We reasoned that this economic pressure along with activity-dependent sorting could induce clustering of neurons with similar responses on an acute basis after brain damage and reorganization. For this reason, and because we found previously that callosal, auditory connections between the non-lesioned hemisphere and the ipsi-lesional AC were shifted laterally in AC (Pallas et al. 1999), we expected that neurons with auditory responses in cross-modal AC would be segregated from those with visual responses. Instead, visual and auditory responses were intermingled throughout the entire AC. It is interesting that the competitive interaction between auditory and visual inputs in terms of proportion of response types did not also affect their spatial distribution within AC. It is possible that microclusters of similarly-responding neurons escaped our detection, or that processes that cause segregation are not operational in cross-modal auditory cortex. At any rate, these results imply that differences in the modality of information carried by the auditory and visual inputs
from MGN during postnatal development are not sufficient to induce segregation or splitting of
the cortical target areas.

**Multisensory neurons in Normal AC**

In addition to auditory neurons, we found multisensory neurons both in normal AC and XMAC.
Previous studies in ferrets and other species have also reported the existence of multisensory
responses in primary auditory cortex, and several investigators have thus begun to question the
degree of modality-specificity in the primary sensory cortices (e.g. Bizley and King 2009;
Ghazanfar and Schroeder 2006). Research on primates (de la Mothe et al. 2006; Lakatos et al.
2007; Smiley et al. 2007) and on rodents and carnivores (Bizley et al. 2007; Campi et al. 2010;
Cappe and Barone 2005; Wallace et al. 2004) has shown that multisensory responses exist in
traditionally-defined primary sensory cortices (cortices with direct thalamic input, which would
include A1 and AAF, defined here as AC). In normal ferrets, King and colleagues reported that
15% of recorded units in A1 and AAF had non-auditory inputs, and these were located primarily
along the outer edges of AC (Bizley and King 2008; Bizley et al. 2007). We encountered a
similar proportion of multisensory to total neurons in normal ferret AC. The multisensory
neurons we recorded in the present study were primarily located at the margins of normal AC,
but particularly in lateroposterior AC, near the border between A1 and the posterior
pseudosylvian and posterior suprasylvian fields. They responded to direct stimulation of the
optic chiasm but not to light, suggesting that they receive only weak visual input. Bizley et al.
(2007) used a more sensitive method of response analysis that included spike timing information,
which may explain why they found greater sensitivity to light. Although we cannot completely
rule out the possibility that some visual units that we recorded were located in non-primary AC, similarly-placed recordings using pure tone stimuli indicate to the contrary.

Multisensory neurons in XMAC

In XMAC the proportion of multisensory neurons was much higher than in normal AC, but it was lower in large lesion animals than in the small lesion group. This was contrary to expectation, because the large-lesion group had more visual input to AC than the small-lesion group, leading to more potential interaction between auditory and visual afferents. One possible explanation is that sensory cortical neurons are more likely to be multisensory when they have weaker cross-modal inputs, perhaps because stronger inputs would outcompete and displace the original modality. Another possibility is that multimodal responsiveness represents an intermediate state between an auditory-dominated normal AC and a visual-dominated XMAC in cases with large lesions.

It is possible that multisensory responses are first created in MGN before reaching XMAC, although previous investigation of retino-MGN projections reported that they were clustered and segregated in small subregions of the ventral MGN, arguing against convergence at the thalamic level (Angelucci et al. 1997; Angelucci et al. 1998; Roe et al. 1993). We propose that the most likely explanation for our data, then, is that new convergences between auditory and visual inputs are made at the level of AC in the lesioned animals.

Although normal AC and XMAC both contain multisensory neurons, our latency data suggest that the origin of visual inputs to these neurons is different in the two cases. The response latency of multisensory neurons to optic chiasm stimulation in XMAC was much shorter than that in normal AC, suggesting that AC in normal animals receives its visual inputs
indirectly from other cortical areas, but that XMAC receives them more directly. Tracer injections made by Bizley and colleagues (2007) in AC of normal ferrets revealed projections from visual cortical regions to AC that may contribute to the multisensory responses seen there. In XMAC however, additional visual inputs come from the retina via the medial geniculate nucleus (MGN) (Sur et al. 1988), and these would be expected to exhibit the shorter latencies that we have seen. The connectional differences between multisensory neurons in normal AC and multisensory neurons in AC of lesioned animals are likely to account for the latency difference, and may result in different response properties as well.

The expanded proportion of multisensory neurons in XMAC is intriguing. Previous perceptual studies on lesioned ferrets with cross-modal visual input to AC argued that they could “see” rather than “hear” visual cues in the rewired auditory cortex (von Melchner et al. 2000). Recent clinical studies showing that thalamic lesions can produce synesthesia (Beauchamp and Ro 2008; Ro et al. 2007) imply that subjects may not be able to identify sensory modalities accurately after cross modal plasticity, however.

In conclusion, the data from this study provide information about the recovery of sensory function after damage to afferent pathways, and suggest a mechanism whereby visual takeover of auditory cortex during cross-modal plasticity might interfere with auditory function through competition for cortical territory. Appropriate and inappropriate inputs can coexist in the affected cortex without strict segregation, and this may not only interfere with efficient processing, but also create barriers to rehabilitation of the compromised modality. Understanding the processes leading to the coexistence of neurons with different functional roles in the affected
cortical areas would be important for designing effective rehabilitation strategies for patients
during recovery.
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Stryker MP, and Harris WA. Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. *J Neurosci* 6: 2117-2133, 1986.


Figure Legends

**Figure 1.** Method for quantification of neuronal response type distribution in auditory cortex. Based on the location of AC on the middle ectosylvian gyrus, we drew an equilateral triangle along the anterior and posterior suprasylvian sulcus and across the tip of the pseudosylvian sulcus (*pss*)(* indicates the tip of *pss*). An internally tangent circle was drawn and divided into four quadrants numbered from one to four as shown. Neurons in these four quadrants were counted and identified by response type (see methods for detail).

**Figure 2.** Assessment of midbrain damage. **A.** An example of a section through the IC. The central nucleus of the inferior colliculus (ICc) was more darkly stained than the surrounding areas (dashed line). **B.** An example of a section through the SC. The superficial layers of SC are marked by a boundary (arrow) that can be recognized under the microscope. **C.** Sketches showing examples of midbrains from one normal and two lesioned animals. Darkened areas show the residual, post-lesion inferior colliculi (IC, top) and superficial layers of the superior colliculi (SC, bottom). The animal with the smaller lesion (center) has some residual SC and IC bilaterally, whereas the animal with the larger lesion (right) is missing left SC and IC entirely.

**Figure 3.** Neuronal response types. **A-D.** Representative post-stimulus response histograms for the different neuronal response types in cross-modal AC. The letter A in the top traces indicates the time course of the auditory stimulus, OX indicates the time of optic chiasm stimulation, and V indicates the timing of the light stimulation. **A.** Auditory neurons respond to sound but not optic chiasm stimulation. **B.** Multisensory neurons respond to both sound and optic chiasm stimulation. **C.** This visual neuron responded to optic chiasm stimulation but not sound. **D.** This
visual neuron was also responsive to light. E-F. The relative proportions of neuronal response
types in AC of E. all normal and F. all lesioned ferrets. A: Auditory neurons. M: Multisensory

Figure 4. Relationship between midbrain lesion type and size and neuronal response type in AC
of experimental animals. A. The proportion of visual neurons was negatively correlated with
midbrain size. B. The proportion of visual neurons was negatively correlated with left SC size.
C. The proportion of visual neurons was not correlated with left IC size. D. The proportion of
auditory neurons was positively correlated with midbrain size. E. The proportion of auditory
neurons was positively correlated with left SC size. F. The proportion of auditory neuron was
positively correlated with left IC size. Each symbol corresponds to one animal (n=14).

Figure 5. Statistical comparison of midbrain size between normal and lesioned animals. Cases
were divided into groups of small and large lesions according to whether they contained AC
neurons that responded to light. The ipsilesional (left) midbrain sizes of lesioned animals in both
the small and large lesion groups were significantly smaller than those of normal animals. (**
represents p<0.001, *** represents p<0.01 (ANOVA)).

Figure 6. Neuronal response types in the three groups. A. The large-lesion group contained
more visual neurons than the small-lesion group. B. The proportion of auditory neurons
decreased with lesion size. C. The proportion of multisensory neurons in the small-lesion group
was significantly higher than that in the normal and large-lesion groups. (** represents
p<0.001, *** represents p<0.01, * represents p<0.05).
**Figure 7.** Reconstruction of locations of recorded neurons in normal AC. Each figure exhibits data from one animal (8 of the 10 cases are shown). Each circle represents one unit with response types as shown in the legend. A indicates auditory stimulation, OX indicates optic chiasm stimulation. The + symbol represents neurons that were responsive to the stimulus, whereas – represents neurons that were not responsive to that modality. A+/OX- indicates responsiveness to auditory stimulation but not to optic chiasm stimulation, A+/OX+ indicates responsiveness to both auditory and optic chiasm stimulation. x indicates a non-responsive site. Scale bar: 1 mm. Arrows at lower right show orientation. M, medial, L, lateral, R, rostral, C, caudal.

**Figure 8.** The distribution of neuronal response types in Normal AC.  
**A.** Pooled data from all 10 normal cases. Each open circle represents one auditory unit. Each gray circle represents one multisensory unit. Acronyms and abbreviations as in Figure 7.  
**B.** The proportion of neurons in each of the four quadrants (pooled data). Auditory stimulus-responsive neurons were uniformly distributed across quadrants but visually responsive neurons were clustered in Q4, in the lateroposterior portion of AC (Chi-square, * indicates p<0.05, *** indicates p<0.001). The dashed line at 25% indicates the value to be expected if response types were evenly distributed.

**Figure 9.** Reconstruction of locations of recorded neurons in AC of the small-lesion group (8 examples from 10 animals are shown). Acronyms and abbreviations as in Figure 7. V+ indicates responsiveness to light stimulation. AOX indicates auditory and OX stimuli were given simultaneously. A+/OX-/AOX- indicates multisensory neurons that did not respond to OX
stimulation alone but whose auditory response could be modulated by it in a suppressive way.

Multisensory neurons increased in frequency in this group but none could be driven by light.

Neurons in the small lesion group defined as visual (A-/OX+/V-) did not respond to sound and responded to electrical stimulation of the optic chiasm but not to light.

**Figure 10.** The distribution of neuronal response types in AC across the population of small lesion cases. Acronyms and abbreviations as defined previously. **A.** Pooled data from all 10 animals. Open circles represent auditory neurons. Gray circles or triangles represent multisensory neurons. Dark triangles represent visual neurons. **B.** The proportion of neurons in each quadrant (pooled data). The distribution of auditory neurons was even across quadrants (Chi-square, p>0.05), but the numbers of multisensory neurons in Q1 and Q4 were significantly different from expected values (Chi-square, ** indicates p<0.01, *** indicates p<0.001), with the numbers significantly higher in Q4 and lower in Q1. Visual neurons were located only in Q3 and Q4. The proportion of visual neurons in Q4 was significantly higher than expected (p<0.01). The dashed line at 25% indicates the value expected if response types were evenly distributed.

**C.** The average distance between single units of each response type in AC. A-A is the average distance between all pairs of auditory neurons. A-M is the average distance between all auditory and multisensory pairs of neurons. M-M is the average distance between all pairs of multisensory neurons. Each symbol represents the mean of average distances for each comparison type from one animal. The average distance between auditory neurons was less than that between multisensory neurons or between auditory and multisensory neurons (ANOVA, * indicates p<0.05).
Figure 11. Reconstruction of locations of recorded neurons in AC of large lesion cases (5 examples from 5 animals are shown). Neurons responsive to light (A-/OX+/V+) were seen in this group (dark circles). Four multisensory neurons (A+/V+) that responded to both sound and light stimuli were recorded in 2 of the animals (F08-201, F09-191) (dark circles with white dot in center indicate A+/V+ neurons; other conventions as in Figs. 7 and 9).

Figure 12. The distribution of neuronal responses in AC of large lesion cases. A. Pooled data from 5 animals. Acronyms and abbreviations as defined previously. B. The proportion of neurons in each quadrant in relation to total recorded auditory, multisensory or visual neurons in AC. Auditory, multisensory and visual neurons are randomly distributed across quadrants (Chi-square, p>0.05). The dashed line at 25% indicates the value to be expected if response types were evenly distributed. C. The average distance between pairs of single units of each response type in AC. V indicates light responsiveness. Each symbol represents the mean of average distances for each comparison type from one animal. The average distance between multisensory and visual neurons was significantly greater than that between pairs of multisensory neurons and between pairs of multisensory and auditory neurons (ANOVA, * indicates p<0.05).

Figure 13. Latencies of responses to optic chiasm stimulation in normal AC and cross-modal AC. The response latency in AC of the small-lesion group (8.76 ±1.03 ms) and in AC of the large-lesion group (5.72 ± 0.65 ms) was significantly shorter than that in normal AC (13.4 ± 1.75 ms). No significant difference was found between small and large-lesion groups. * indicates p<0.05 and *** indicates p<0.001 (ANOVA).
Figure 14. Schematic of possible inputs to normal AC, to XMAC of small-lesion animals, and to XMAC of large-lesion animals. The gray scale to the right provides the key to the gray levels used in lines and structures in the drawings. The letter A represents auditory and V represents visual, with intermediate gray levels corresponding to degrees of multisensory responsiveness.

A. Normal connectivity pattern. B. The small, dark circle in this left side lateral view of Normal AC represents a cluster of multisensory neurons in the auditory field (large light gray circle). C. Dashed line indicates the rewiring of retinal axons to the left MGN. The left AC then receives reduced auditory input and ectopic visual input from MGN. White ovals in left and right IC and left SC indicate neonatal, partial lesion of these midbrain structures. The spared right IC may provide auditory input to the left MGN after the lesion. The narrow lines from left ear to spared IC indicate preserved auditory projections. D. The AC of small-lesion animals has increased visual responsiveness and decreased auditory processing. The darker, small circle in AC represents clustered multisensory neurons. The extent of clustering is smaller in XMAC than in normal AC. E. The XMAC of large lesion animals has the highest proportion of visual neurons in the three groups of animals. No clustering of multisensory neurons was seen in AC of large-lesion animals.
Table 1. The proportion of neuron types and residual midbrain volumes in lesioned animals.

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<th>Multisensory (%)</th>
<th>Visual (%)</th>
<th>L-IC (%)</th>
<th>R-IC (%)</th>
<th>L-SC (%)</th>
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<td>31.58</td>
<td>0</td>
<td>47.86</td>
<td>66.59</td>
<td>62.90</td>
<td>77.31</td>
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<tr>
<td>Large lesion</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>07-61</td>
<td>35</td>
<td>10</td>
<td>55</td>
<td>0</td>
<td>55.36</td>
<td>3.89</td>
<td>69.59</td>
</tr>
<tr>
<td>07-107</td>
<td>26.33</td>
<td>28.95</td>
<td>44.74</td>
<td>21.38</td>
<td>63.78</td>
<td>0</td>
<td>35.48</td>
</tr>
<tr>
<td>08-201</td>
<td>45.45</td>
<td>40.91</td>
<td>13.64</td>
<td>0</td>
<td>34.69</td>
<td>5.44</td>
<td>93.59</td>
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<tr>
<td>09-21</td>
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<td>14.29</td>
<td>38.14</td>
<td>0</td>
<td>37.70</td>
<td>6.26</td>
<td>82.54</td>
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<tr>
<td>09-191</td>
<td>64.71</td>
<td>17.65</td>
<td>17.65</td>
<td>5.62</td>
<td>23.59</td>
<td>11.48</td>
<td>55.65</td>
</tr>
</tbody>
</table>

The midbrain volumes were normalized to average midbrain volumes of normal animals. IC represents inferior colliculus, SC represents superior colliculus. L- Represents left. R- Represents right.
Normal Group

Small lesion Group

Large lesion Group
(A) Normal

(B) Lesioned

(C) Auditory neuron

(D) Multisensory neuron

(E) Visual neuron

(F) Visual neuron (light responsive)
Normalized Left Midbrain Volume

- Normal Group
- Small lesion Group
- Large lesion Group

Significance levels:
- **: p < 0.01
- ***: p < 0.001
Auditory Neuron A+/OX-
Multisensory Neuron A+/OX+
Non-responsive penetration
**A**

A+/OX-  A+/OX+

**B**

Auditory Multisensory

% Neurons in Each Quadrant (pooled data)

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>Auditory</th>
<th>Multisensory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
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</tr>
<tr>
<td>Q2</td>
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<tr>
<td>Q3</td>
<td></td>
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</tr>
<tr>
<td>Q4</td>
<td></td>
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</tr>
</tbody>
</table>

25%  

***

*
Auditory Neuron A+/OX-
Multisensory Neuron A+/OX+
Multisensory Neuron A+/OX-/AOX-
Visual Neuron A-/OX+/V-
Non-responsive penetration

○ Auditory Neuron A+/OX-
○ Multisensory Neuron A+/OX+
△ Multisensory Neuron A+/OX-/AOX-
▲ Visual Neuron A-/OX+/V-
× Non-responsive penetration
- Auditory Neuron A+/OX-
- Multisensory Neuron A+/OX+
- Multisensory Neuron A+/OX-/AOX-
- Multisensory Neuron A+/V+
- Visual Neuron A-/OX+/V-
- Visual Neuron A-/OX+/V+
- Non-responsive penetration
B

% Neurons in Each Quadrant (pooled data)

Auditory  Visual  Multisensory

C

Normalized Distance

A-A  A-M  A-V  M-V  V-V  M-M
Latehncy to Optic Chasim Stimulation (ms)

- Normal Group
- Small lesion Group
- Large lesion Group

Comparison: Normal Group vs. Small lesion Group

Significance:
- *: p < 0.05
- ***: p < 0.001
Normal Projection

Xmodal Projection (Possible source of auditory inputs)

AC of small lesion animal

AC of large lesion animal