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Title

Effects of chronic cochlear electrical stimulation after an extended period of profound deafness on primary auditory cortex organization in cats

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Abstract

Extended periods of deafness have profound effects on central auditory system function and organization. Neonatal deafening results in loss of the normal cochleotopic organization of the primary auditory cortex (AI), while environmentally derived intra-cochlear electrical stimulation, via a cochlear implant, initiated shortly after deafening, can prevent this loss. We investigated whether such stimulation initiated after an extended period of deafness can restore cochleotopy. In two groups of neonatally-deafened cats a multi-channel intracochlear electrode array was implanted at eight weeks of age. One group received only minimal stimulation, associated with brief recordings at 4-6 week intervals, over the following 6 months to check the efficacy of the implant. In the other group, this 6-month period was followed by 6 months of near-continuous intra-cochlear electrical stimulation from a modified clinical cochlear implant system. We recorded multi-unit clusters in the auditory cortex and used two different methods to define the region of interest in putative AI. There was no evidence of cochleotopy in any of the minimally stimulated animals, confirming our earlier finding. In three of six chronically stimulated cats there was clear evidence of AI cochleotopy, and in a fourth cat in which the majority of penetrations were in the anterior auditory field there was clear evidence of cochleotopy in that field. The finding that chronic intra-cochlear electrical stimulation after an extended period of deafness is able to restore cochleotopy in some (but not all) cases, has implications for the performance of patients implanted after an extended period of deafness.

Introduction

Cochlear implants have been used to provide functional hearing to over 220,000 individuals with profound/severe sensorineural hearing loss (SNHL). The loss of afferent input associated with a neonatal SNHL has effects on the morphology and function of neurons along the auditory pathway, and thus on the functional organization of the central auditory system, which influence the effectiveness of a subsequently introduced cochlear implant (for reviews see Shepherd & Hardie, 2001; Fallon *et al.*, in press).

One of the fundamental organizational features of the central auditory system is its cochleotopic organization, i.e., the fact that near-threshold activation at a particular cochlear locus results in activation of a restricted region of a given nucleus or cortical region, and that the cochlea is topographically represented (or "mapped") in that structure. This cochleotopy is the basis of the tonotopic organization of the lemniscal auditory system in hearing animals. When a normal-hearing adult cat is acutely deafened and auditory nerve fibers innervating discrete regions of the cochlea are activated electrically, neurons in the primary auditory cortex (AI) respond with lowest threshold to a particular cochlear electrode, the "best electrode" (BE) for that cortical site. The spatial distribution of BEs across the AI is cochleotopic, corresponding to the tonotopy seen in hearing cats, with neurons in rostral areas of the AI having BEs in the basal cochlea and neurons at progressively more caudal locations in the AI having BEs at progressively more apical cochlear locations (Raggio & Schreiner, 1999; Fallon *et al.*, 2009). We have previously reported that this cochleotopy was absent in neonatally deafened cats in which cortical responses to intracochlear electrical stimulation (ICES) were examined 7-13 months after deafening (Fallon *et al.*, 2009), and others have similarly reported weak or no signs of tonotopy after a long-term SNHL (Dinse *et al.*, 1997; Raggio & Schreiner, 1999). However, we found that cochleotopy was normal in neonatally deafened cats that received chronic, environmentally derived ICES for periods of 3-7 months (Fallon *et al.*, 2009). The chronically stimulated cats in that study were implanted at ~8

weeks of age, and chronic stimulation was initiated ~14 days after surgery. It is therefore unclear whether cochleotopy was still present at the time of the initiation of stimulation and simply maintained by the chronic ICES, or whether it had been lost at that time and was restored by the stimulation. The present experiment was designed to establish unequivocally whether chronic ICES is able to restore lost cochleotopy by examining the cochleotopic organization of the AI in neonatally-deafened cats which had experienced an extended period of deafness – shown to be long enough for cochleotopy to have been lost – before chronic stimulation was initiated. The ability of chronic ICES to restore aspects of central auditory processing and functional organization degraded by an extensive period of deafness is of obvious clinical relevance to those cases where an implant is provided to a patient after an extended period of early-onset deafness. Preliminary findings have been presented in abstract form (Fallon *et al.*, 2010a; b).

Materials and Methods

Ten healthy cats (bred in the Institute's animal facility; 6 female; 4 male; details given in Table 1) with otoscopically normal tympanic membranes were used in the present study. The basic methods were as described in Fallon et al. (2009), and these methods will therefore be described only briefly here. All procedures were in accordance with Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and with the Guidelines laid down by the National Institutes of Health in the US regarding the care and use of animals for experimental procedures, and were approved by the Royal Victorian Eye and Ear Hospital Animal Research and Ethics Committee.

Deafening procedure

Each cat was administered a daily subcutaneous (s.c.) injection of neomycin sulfate (60 mg/kg) from one day after birth for seventeen days (Leake *et al.*, 1991). Hearing status

was then measured, and if the animal was not profoundly deaf neomycin injections were continued in three-day increments until the animal was profoundly deaf. The criterion of profound deafness was the absence of a monaural click-evoked auditory brainstem response (ABR) at 93 dB peak equivalent sound pressure level in either ear. ABR-recording methods were as described previously (Coco *et al.*, 2007).

Cochlear implantation and chronic stimulation

At approximately eight weeks of age, the cats were unilaterally implanted (left cochlea) with an eight-ring scala tympani electrode array and lead-wire assembly, using previously published techniques (Coco *et al.*, 2007). Briefly, surgery was performed under aseptic conditions, with each animal premedicated using acepromazine maleate/atropine sulphate (0.05 ml/kg s.c.) and maintained at a surgical level of anesthesia using a closed circuit anesthetic machine delivering a mixture of halothane and oxygen. The bulla cavity was opened and flushed with amoxicillin (10 mg/ml), and the round window membrane was incised. The array was inserted 8 mm into the scala tympani, placing the most apical electrode (E1) at the ~10-kHz place and the most basal electrode (E8) at the ~26-kHz place (Brown *et al.*, 1992), and the round window was sealed with crushed muscle. An extra-cochlear ball electrode was placed under the temporalis muscle to allow for monopolar stimulation. The leadwire was fixed at the bulla and on the dorso-lateral part of the skull, before passing subcutaneously to exit the body through an incision at the nape of the neck.

Approximately two weeks after surgery, and every 4-6 weeks thereafter, the animals were anesthetized with ketamine and xylazine (20 mg/kg i.m., 2 mg/kg s.c.) and an electrically evoked ABR (EABR) was recorded for each stimulating electrode using standard electrophysiological techniques (Coco *et al.*, 2007). Optically isolated biphasic current pulses were generated under computer control and delivered to the intracochlear electrode array. Responses were recorded differentially using the same techniques as for the ABR described

previously (Fallon *et al.*, 2009). Two recordings were made at each current level and current amplitude was reduced to levels below threshold, defined as the current level required to evoke a peak-trough response amplitude of at least 0.2 μV for wave IV of the EABR (a latency window of 2.4-2.9 ms following stimulus onset) for both responses. In addition to the EABR recordings, intracochlear electrode impedances were measured each week using Custom Sound® to monitor the status of the implanted electrode array.

At eight months of age, these cats were randomly allocated to one of two groups. For four animals, the functional organization of putative AI was examined in an acute experiment. The only ICES experienced by this group was that involved in the testing of EABRs, and they will therefore be referred to as the "minimal stimulation" (MS) group. For members of the second, "delayed stimulation" (DS) group (6 cats), a chronic stimulation program was initiated and continued for 6 months. Each animal received unilateral simulation at multiple (typically 8, depending on the number of functional electrode contacts) sites within the lower basal turn from a Nucleus® CI24 cochlear implant and Nucleus® ESPrit 3G speech processor as described previously (Fallon *et al.*, 2009). The stimulator package was carried in a harness worn by the unrestrained animal, and the output of the stimulator was directly connected to the leadwire assembly. The environmentally derived stimulation was presented continuously except for occasional periods when flat batteries had to be replaced. The speech processors were programmed using standard clinical frequency allocation tables and delivered stimulation at 500 pulses per second per electrode at stimulus levels from 3 dB below to 6 dB above the EABR threshold. Monopolar (MP) stimulation was used initially in all animals, but in 3 cases it was necessary to shift to common-ground (CG) electrode configurations in the course of the chronic stimulation period, as MP stimulation was eliciting muscle twitches. This change was not associated with any other measurable change (i.e. EABR threshold or electrode impedance). For MP stimulation, each biphasic current pulse had a 25-μs phase interval and an 8-μs inter-phase gap, while for CG stimulation each

biphasic current pulse had a 100-μs phase interval and a 50-μs inter-phase gap. The different pulse durations reflect the greater efficacy of MP stimulation, although both configurations are used clinically (Seligman & Shepherd, 2004). These stimulus levels were assessed by monitoring behavioral characteristics (orienting responses, including head and pinna movements) for each animal, and were confirmed to elicit behavioral responses and to cause no discomfort. Both self-vocalizations and vocalizations by other animals housed in the facility produced changes in the stimulus levels that were within the perceivable range for each animal. Similarly, other environmental sounds associated with the normal running of such a facility would be expected to have also been perceivable, and this was confirmed by observation of responses to environmental events.

Cortical recording and data analysis

At either 9-10 (MS group) or 14-15 (DS group) months of age (see Table 1), acute electrophysiological experiments were performed to record the response properties of auditory cortical neurons and determine the cochleotopic organization of putative AI. Anesthesia was induced with ketamine and xylazine (20 mg/kg i.m., 2 mg/kg s.c.) and a tracheal cannula was inserted. Sodium pentobarbitone (intravenous) via a slow-infusion pump (0.3 - 0.7 mg kg^{-1} h^{-1}) was used to maintain a steady light level of surgical anesthesia throughout the recording period. Heart rate, respiration rate, end-tidal CO2, and core body temperature were maintained within normal levels. Animals were placed in a stereotaxic apparatus in a sound-attenuated Faraday room, and a craniotomy was performed to expose the right auditory cortex (i.e., that contralateral to the implanted cochlea). The dura mater was removed, and a calibrated photograph was taken of the AI and surrounding cortex. Single- and multi-unit recordings were made from putative AI using a combination of single tungsten micro-electrodes (WPI; Sarasota, Florida), and linear (A1x32-6mm-100-413-A32, NeuroNexus Technologies; Ann Arbor, Michigan) and planar (Cyberkinetics; Foxborough,

Massachusetts) silicon arrays. Although the precise location of the AI varies with respect to sulcal landmarks **(Merzenich *et al.*, 1975; Reale & Imig, 1980)**, the larger part of the field in all cats is located on the middle ectosylvian gyrus and in the rostral bank of the posterior ectosylvian sulcus (PES). The planar silicon array was always inserted in the middle ectosylvian gyrus and therefore the size of the array varied based on the sulcal patterning of the cortex (i.e. in the majority of animals a 7x7 array was used, while in some animals smaller 6x6 arrays were necessary). Additional microelectrode penetrations were made in the gyrus dorsal of the array and between the array and the ectosylvian sulci. The Cyberkinetics electrodes were 1.0 mm in length, and recordings from the tungsten microelectrodes were typically made at depths from 600 – 1800 μm, so most recordings were made from the middle (thalamorecipient) cortical layers. In most cats, an attempt was made to record from the low-frequency region of the AI in the rostral bank of the PES by making recordings at a range of depths in long penetrations (with tungsten microelectrodes and/or NeuroNexus arrays) down the sulcal bank. Recording locations were marked on the photograph relative to the vascular landmarks. Single- and multi-unit recordings were captured at a sample rate of 30 kHz using the Cerebus system (Cyberkinetics; Foxborough, Massachusetts) and single- or multi-unit recordings were identified off-line using standard spike discrimination techniques in IgorPro (Wavemetrics; Lake Oswego, Oregon). A recording site was classified as responsive to ICES (Table 1) if stimulation produced an increase in firing of at least 0.5 spikes/s. Acute experiments were of approximately 40 hours duration and at the end of the experiment the animals were terminated with an overdose of sodium pentobarbital (150 mg/kg, intravenous). The cochleae were then dissected out and the insertion depth of the electrode array at its original implantation position within the cochlea confirmed.

To characterize the basic characteristics of auditory cortical neuronal responses to ICES, a range of stimulus currents, on a range of intracochlear electrodes, was utilized. Input-output functions for each stimulating electrode were determined with a randomized stimulus

matrix that consisted of currents from 0 to up to 2 mA (or the maximum level that did not induce non-auditory responses, typically muscle contractions) on all stimulating electrodes, presented at a rate of up to 1.5 stimuli per second. Each input-output function was fitted with a saturating Gaussian function (Sachs & Abbas, 1974), from which the threshold (defined as the current required to achieve a half maximal response), an estimate of the error in determining the threshold, and dynamic range (defined as the current range required to achieve an increase in response from 10% to 90% of maximal) for each stimulating electrode could be determined. For each recording site, the BE (the analogue of characteristic frequency) was defined as the stimulating electrode with the lowest normalized threshold. It was necessary to normalize cortical thresholds to EABR threshold for that electrode configuration, as stimulation mode, hearing status and stimulating electrode location can all alter threshold (Fallon *et al.*, 2009). If the thresholds on two adjacent stimulating electrodes were not significantly different, the average of the two electrodes was assigned as BE. If the thresholds of three or more adjacent stimulating electrodes, or two non-adjacent stimulating electrodes, did not differ, the site was defined as being "broadly tuned" and was excluded from subsequent analysis. The "depth of tuning" (an analog of acoustic bandwidth) at each site was defined as the difference in threshold, expressed in dB, between BE and the two adjacent electrode thresholds.

The effects of minimal ICES and of delayed chronic ICES following a long-term SNHL on the cochleotopic organization of the AI were quantified in two ways. First, a cortical map of the variation in BE across putative AI was constructed. The BE maps were analyzed for a correlation (Pearson Correlation) between BE and caudorostral location in the AI (approximately parallel to the main tonotopic axis in normal-hearing animals). Additionally, in those cases in which cochleotopy was present in AI, the cochlea-to-cortex mapping ratio (i.e., the shift across the cortex corresponding to a 1-mm shift along the basilar membrane) was determined.

Detailed analysis of BE maps was initially limited, as per Fallon et al. (2009), to the region between the lip of the rostral bank of PES and a point on the gyrus 2 mm caudal to the anterior ectosylvian sulcus (AES). In a normal-hearing cat, this region typically spans a characteristic-frequency range from 5–10 kHz near the lip of PES to the high-frequency edge of the AI near AES (Reale & Imig, 1980), matching the frequency range/place of our intracochlear stimulating electrode array. However, a characteristic-frequency reversal, indicating the transition from the AI to the anterior auditory field (AAF), which has a tonotopic organization that is approximately a mirror-image of that in the AI, can occur within this region (Knight, 1977; Reale & Imig, 1980; Phillips & Irvine, 1982; Imaizumi *et al.*, 2004). Such a reversal was evident in the BE maps in a number of animals (e.g., cat 978; Figure 2). Therefore, an alternative method of defining the region of interest was developed. For all BE maps, both a single straight line and a 'broken stick' model (i.e. two straight lines of opposite slope than meet at a common point) were fit to the data for all recording sites. If the broken stick model was a better fit than a single straight line (based on the sum of the residuals), the reversal point of the model was taken to be the AI-AAF border. The correlation analysis was repeated using all recording locations caudal to the AI-AAF border or all locations if no AI-AAF border was found. For convenience, these two analyses will be referred to as the "restricted-region" and the "best-fit" analyses.

The organization of the auditory cortex was also assessed by measuring the cortical spread of activation, defined as the area of cortex that was activated by a stimulus 2 dB above the minimum cortical threshold for a particular intracochlear electrode (Fallon *et al.*, 2009). To calculate the cortical areas activated, Voroni tessellation was used to create tessellated polygons, with all recordings sites at their centers (Bao *et al.*, 2003). The Voroni tessellation was done using all recording sites, not just sites at which it was possible to determine a BE, and was bounded by a rectangular box encompassing all recording sites **(Fallon *et al.*, 2009)**.

In this way, every point in the cortex could be assigned a threshold derived from a sampled cortical site that was closest to this point.

Because of the unmatched design of the experimental cohorts (i.e. no minimal stimulation CG group), the effects of mode of stimulation in the DS group, and the differences between groups with respect to the effects of chronic monopolar ICES on the basic response properties of AI units, were assessed with t-tests.

Results

Consistent with our previous report (Fallon *et al.*, 2009), EABR thresholds exhibited a complex dependence on stimulation mode and stimulating electrode position. Also consistent with previous work (Shepherd *et al.*, 1994), electrode impedances were relatively stable over the implantation period and no animal needed to be removed from the study due to implant or electrode failure.

Basic Response Characteristics

The number of recording sites in each animal at which multi-unit responses exhibited a short-latency (less than 40 ms) response to ICES are shown in Table 1. There was no difference in the quality of recordings between the groups, with clearly identifiable multi-unit activity present on recordings from both the single tungsten electrodes and the linear and planar arrays. Off-line spike discrimination techniques were able to isolate a total of 143 single-units from the multi-unit activity, of which 19 were responsive to electrical stimulation and 124 were unresponsive, exhibiting only spontaneous activity. This proportion might appear small, but is in accordance with recent evidence that sensory cortices contain sub networks of "rare high-responsive and stimulus–driven neurons" embedded in a sea of relatively unresponsive cells (Lutcke *et al.*, 2013). The average spontaneous firing rate for the single-units was less than 1 spike/s, and there was no difference between the experimental

groups (Student's *t*-test: t_{122} = -0.33, P = 0.74). The data for driven single-units exhibited the same trends evident in the multi-unit data presented below, but because of the low numbers separate statistical analysis of these data was not appropriate.

Consistent with our previous report (Fallon *et al.*, 2009), there was a monotonic increase in the number of spikes elicited with increasing charge level that was well approximated by a sigmoidal function. It was possible to determine a 'best electrode' at approximately 85% of all responsive recording sites (see Table 1). As summarized in Table 2, the BE threshold (charge required to produce half maximal response) for MP stimulation was lower than that for CG stimulation in the DS group (Student's *t*-test: t_{250} = 6.6, P < 0.001). BE thresholds for MP stimulation for the MS group was significantly lower than that for the DS group (Student's *t*-test: t_{238} = -2.0, P = 0.047). Dynamic range (charge change required to increase the response from 10 to 90 % of maximal response) for MP stimulation was lower than that for CG stimulation in the DS group (Student's *t*-test: t_{243} = -6.0, P < 0.001). Dynamic range for MP stimulation was also lower in the DS group than in the MS group (Student's *t*-test: t_{222} = 2.9, P = 0.0041). Finally, the depth of tuning (the increase in threshold from BE to the adjacent stimulating electrode) for MP stimulation was lower than that for CG stimulation in the DS group (Student's *t*-test: t_{227} = -7.6, P < 0.001). The depth of tuning for MP stimulation was also lower in the DS group than in the MS group (Student's *t*-test: t_{221} = 5.8, P < 0.001).

Cochleotopic Organization

Representative BE maps of putative AI for a MS and a DS animal are illustrated in Figure 1. As indicated in Methods, the initial "restricted region" analysis was restricted to data from recording sites between the lip of the rostral bank of PES and a point on the gyrus 2 mm caudal to AES (see vertical broken lines in C and F). The correlation between BE and caudorostral position for the MS animal was not significantly different from zero (r_{30} = 0.23,

P = 0.25). In contrast, in the DS animal there was a clear cochleotopic organization, as reflected in a significant positive correlation (r_{36} = 0.62, P < 0.001) between BE and caudorostral position, and a cochlea-to-cortex mapping of 1.2 (i.e. a 1-mm shift along the basilar membrane corresponded to a 1.2-mm shift across the cortex).

The BE maps for all animals are illustrated in Figure 2, and the mean correlations and cochlea-to-cortex mapping ratios are presented in Figure 3A & B (open bars). In the restricted-region analysis, the correlation between BE and caudorostral position was not significantly different from zero for any of the MS animals (individual correlation coefficients in Figure 2; all p-values > 0.05) i.e. there was a complete lack of cochlea-to-cortex mapping. The mean (± SEM) correlation for the group was 0.08 ± 0.16 (Figure 3A), which was also not significantly different from zero (*t*-test: t_3 = 0.47, P = 0.67). The restricted-region analysis of the DS animals revealed significant positive correlations for three animals (viz., 973, 975 and 976; correlations of 0.44, 0.50 and 0.62 respectively; p-values < 0.01; p-values for other cases > 0.05); and the group mean (± SEM) correlation for the DS group was 0.32 ± 0.11, which was significantly greater than zero (*t*-test: t_5 = 2.9, P = 0.033). The mean (± SEM) cochlea-to-cortex mapping for the three cats with significant correlations was 1.6 ± 0.4 mm/mm.

Although the restricted-region analysis yielded significant correlations for only three of the DS animals, inspection of the data for the other three animals in the group (viz., 978, 979 and 980) suggests the possibility of cochleotopic organisation, with a BE reversal corresponding to the AI-AAF border. To determine objectively if a BE reversal was present, the BE map data for both groups were fit with both a single straight line and a broken stick model. The solid lines in Figure 2 illustrate the fit of the model with the lowest sum of residuals. All of the MS animals were best approximated by the single straight line (i.e. there were no evidence of BE reversals). In contrast, four of the DS animals (viz., 973, 978, 979 and 980) were best approximated with a broken stick model, indicating that the recordings

most likely straddled the AI-AAF border. As stated in the Methods, the correlation analysis for all animals was repeated using recording locations caudal to the reversal point (the putative AI-AAF border), or all locations in the cases with no reversal. The individual correlations obtained using this best-fit analysis are presented in Figure 2, and the mean correlations in Figure 3A & B (filled bars). As with the restricted-region analysis, none of the MS animals exhibited a significant correlation (all p-values > 0.05) with a mean (± SEM) correlation of 0.02 ± 0.10, which was not significantly different from zero (*t*-test: $t_3 = 0.17$, $P = 0.87$). In contrast, the three DS animals for which the restricted region analysis yielded a significant positive correlation also had a significant positive correlation in the best-fit analysis (cats 973, 975 and 976; correlations of 0.61, 0.45 and 0.80 respectively; p-values < 0.01), with mean (± SEM) cochlea-to-cortex mapping of 1.9 ± 0.2 mm/mm (which was not significantly different from the restricted-region value; Student's *t*-test: $t_4 = 0.99$, $P = 0.38$). In the two other DS animals for which the majority of points were in putative AI (viz., cats 978 and 979), the correlations were positive but non-significant. In cat 980, the majority of the recording sites were rostral of the inflection point in the brokenstick fit, and thus appeared to lie in the AAF rather than the AI. In support of this interpretation, there was a significant negative correlation ($r_{64} = -0.34$, P = 0.022) if only recording sites rostral to the inflection point (the putative AI-AAF border) were analysed, with a cochlea-to-cortex mapping of -2.4 mm/mm. The group mean (± SEM) correlation for points designated as being in the AI for the DS group was 0.42 ± 0.10, which was significantly greater than zero (*t*-test: $t_5 = 4.0$, $P = 0.010$). When cat 980, for which there were very few data points in putative AI, was removed from this analyis, the mean AI correlation was 0.48 ± 0.10, which was significantly greater than zero (*t*-test: $t_4 = 4.8$, $P = 0.009$).

Examination of the electrical stimulation characteristics (e.g., number of active electrodes, dynamic range) did not reveal any systematic differences between those animals in the DS group that exhibited restoration of cochleotopy and those that did not. There was

also no obvious differences in experimental treatment (i.e. threshold, electrode impedance, electrode location) between the DS animals that exhibited organization and those that did not.

As previously reported (Fallon *et al.*, 2009), as the stimulus current increased from near threshold to supra-threshold levels, the area of auditory cortex activated by the stimulus increased monotonically, with a near-linear increase over the first 2 dB, for all animals. Using the area activated at 2 dB above minimal cortical threshold resulted in a cortical spread of activation of 11.0 ± 1.3 mm^2 and 8.4 ± 1.1 mm^2 for the MS and DS groups, respectively (Figure 3C). This difference in cortical spread was not significant (Student's *t*-test: t_{68} = -1.26, P = 0.21).

Discussion

The major finding of this study is that chronic environmentally derived ICES is at least partially (i.e. in some but not all animals) able to restore the cochleotopic organization of the AI that is lost as a consequence of an extended period of SNHL following neonatal deafening. In the following sections, the findings relating to the effects of deafness and of chronic ICES will be discussed in the context of previous results, and the clinical implications of the results will be considered in the context of the limited analogous data available for human cochlear implantees.

Effects of deafness

The complete lack of cochleotopy in AI, as defined by either the "restricted-region" or "best-fit" analyses, in cats in the MS group confirms our previous finding in cats that were neonatally deafened and acutely implanted with cochlear stimulating electrodes at the time of cortical recording (i.e., at ~7-13 months of age). It is also in agreement with other reports that cochleotopy is weak or absent after a long-term SNHL in ototoxically deafened cats (Dinse *et al.*, 1997; Raggio & Schreiner, 1999). Hartmann et al. (1997) suggested that the variation in threshold of field potentials evoked by electrical stimulation via a basal and an apical pair of

electrodes in congenitally deaf white cats was indicative of a rudimentary level of cochleotopic organization in at least some animals. However, there does not appear to have been a detailed multi- or single-unit examination of cochleotopy in these cats.

The complete loss of cortical cochleotopy as a consequence of neonatal deafness would be surprising if cochleotopy were simply a consequence of topographically ordered projections from the cochlea to the auditory cortex. However, it is now well established that the highly convergent and divergent nature of the projections between and within central auditory system structures (e.g. Irvine, 1986) is such that AI neurons receive input derived from a wide range of cochlear regions other than that providing their characteristic frequency input (e.g. Phillips & Hall, 1992; Metherate *et al.*, 2005). In normal hearing animals, the sharp frequency tuning of AI neurons and the resultant tonotopic organization is established and maintained by complex integration of excitatory and inhibitory inputs. It is presumably these integrative processes that are lost as a consequence of long-term deafness and at least partially restored by the restoration of patterned input to the central auditory system by environmentally derived ICES. In this context, it is of interest that long-term deafness does not result in loss of cochleotopy in the inferior colliculus of neonatally deafened cats (Vollmer *et al.*, 2007). This difference between the inferior colliculus and the AI presumably reflects the fact that collicular cochleotopy is more dependent on orderly projections from the cochlea and less on neural integrative processes than that in AI.

Effects of chronic ICES

In our previous study (Fallon *et al.*, 2009) we found that cochleotopy was normal in neonatally deafened cats that received chronic, environmentally derived ICES for periods of 3-7 months, initiated at approximately 10 weeks of age (i.e., ~14 days after implantation at ~8 weeks of age). A secure tonotopic gradient has been described in the AI of hearing kittens as young as ten days of age (i.e., at P10) (Eggermont *et al.*, 1993; Bonham *et al.*, 2004).

Although no recordings have been made in younger kittens, it is likely that cochleotopy is established well before P10 and therefore before completion of deafening in our studies. It is unclear, however, whether this cochleotopy was lost in the short period (8-9 weeks) of deafness prior to the initiation of stimulation in the Fallon et al. (2009) study. It is therefore uncertain whether chronic ICES had simply maintained existing cochleotopy in that study or whether it was able to restore lost cochleotopy. Although the present experiment has not resolved that uncertainty, it has established that chronic ICES is in fact able to restore cochleotopy lost after an extended period of deafness. ICES in cats in the DS group was initiated after 8 months of profound SNHL, a period of deafness which had resulted in loss of cochleotopy in all animals in the MS group in this study and in the long-term deaf unstimulated animals in our previous study. The fact that cochleotopy was present in some of the animals in our DS group therefore clearly demonstrates that chronic ICES is capable of restoring cochleotopy even when initiated after a long period of deafness. As noted in the Results, there were no obvious differences in experimental treatment (i.e. threshold, electrode impedance, electrode location) between those cats in the DS group that exhibited cochleotopy and those that did not.

Comparison of these results with those of Fallon et al. (2009) indicates that chronic ICES initiated after a long period of profound SNHL is less effective than stimulation initiated in young animals shortly after deafness. In the present study, cochleotopy was found to be present in AI on the basis of both the restricted-region and best-fit analyses in three animals. In one cat in which the best-fit model indicated that the majority of recordings were in the AAF rather than the AI, there was also evidence of cochleotopy in the significant negative correlation between BE and caudorostral position in the cortex. Given that tonotopy in the AAF or normal hearing animals is mirror-image reversed relative to that in AI, the negativity of this correlation confirms the identification of the AI – AAF border by the broken-stick model, and its statistical significance indicates that AAF is cochleotopically

organized. Although there is no evidence indicating whether cochleotopy is lost in AAF with deafness, it would seem almost certain that if cochleotopy is lost in the AI it would also be lost in the AAF. This cat can therefore reasonably be considered a fourth case in which cochleotopy was restored by chronic ICES. Thus four of the six chronically stimulated animals in the present study exhibited cochleotopy. In contrast, all seven of the chronically stimulated animals in Fallon et al. (2009) study exhibited cochleotopy. In accordance with this difference, the mean correlations between BE and caudorostral position using the restricted-region analysis of AI (i.e., the analysis common to the two studies) were 0.32 in the present study and 0.51 in Fallon et al. (2009)). Although the mean correlation in the present study is undoubtedly reduced somewhat by the inclusion of data from cat 980 in which little data were obtained from AI, the differences in both the proportion of animals exhibiting cochleotopy and the magnitude of the correlations strongly suggest that chronic ICES is more effective in either maintaining or restoring cochleotopy when it is initiated after a short period of deafness in young animals than it is in restoring cochleotopy after an extended period of deafness in older animals.

A number of factors might underlie this difference, and the evidence from the two studies does not allow them to be differentiated. If cochleotopy had simply been maintained in the chronically stimulated animals in the Fallon et al. (2009) study, it could simply be that such stimulation is more effective in maintaining cochleotopy than in restoring it. Alternatively, if cochleotopy had been lost in those animals, it could be that the extent of the changes underlying that loss was more limited after a short period of deafness than after the long period of deafness experienced by the animals in the present study. Alternatively or additionally, it could be that the greater plasticity in the auditory system of the young animals in that study, in which stimulation was initiated at ~10 weeks of age, resulted in better restoration than the more limited plasticity in our much older animals in which stimulation was initiated at 8-9 months of age. In accord with this suggestion, Kral et al. (2002; 2006)

have presented evidence for a sensitive period between the second and sixth months of life in the congenitally deaf cat.

Our results are in accord with other evidence that chronic ICES initiated in mature animals can restore aspects of auditory cortical organization and processing degraded as a consequence of long-term deafness. Vollmer and Beitel (2011) showed that temporal processing in the AI was degraded in neonatally-deafened cats that had been deaf for periods in excess of 3 years, but that a number of features of temporal processing were restored by some months of behaviourally relevant ICES after such long-term deafness.

Our data provide no indication of the time course of the changes in cortex that underlie the restoration of cochleotopy in those cats in which it is restored. We are currently examining this issue in neonatally deafened cats in which chronically implanted cortical electrode arrays allow cortical responses to ICES to be measured at repeated intervals over the period of stimulation (Irvine *et al.*, 2013).

The other respects in which our two groups differed were that dynamic range and depth of tuning (for MP stimulation) were significantly greater, and thresholds significantly lower, in the MS than in the DS group (Table 2). In contrast, in Fallon et al.'s (2009) study, depth of tuning for MP stimulation was significantly greater in the chronically stimulated than in the long-term deaf unstimulated group, but dynamic range and threshold did not differ between the two groups. The thresholds for both MS and DS groups were approximately 0.6 dB lower in the current study, compared to the equivalent groups in the Fallon et al. (2009) study; however the reason for the reduced thresholds is not clear. Cats in the current MS group and those in Fallon et al.'s long-term deaf unstimulated group had similar periods of deafness, and the fact that the values for dynamic range (2.9 ± 0.3 and 2.8 ± 0.2, respectively) and depth of tuning (1.3 ± 0.1 and 0.9 ± 0.1 respectively) were very similar in those two groups supports the conclusion from the cochleotopy data that the minimal stimulation in the MS group had no substantial effect on these parameters. The difference between the two

studies with respect to the effects of chronic stimulation suggests that the restoration of cochleotopy by chronic ICES in the adult animals in the current study occurred at the cost of the precision of electrical tuning at a given cortical site, whereas the maintenance or restoration of cochleotopy by chronic ICES in the young animals in Fallon et al.'s (2009) study did not. It is not clear why this should be the case.

Clinical implications and comparison with human data

The fact that chronic ICES initiated in mature animals after a prolonged period of profound neonatal deafness can restore aspects of auditory cortical functional organization and processing degraded by deafness has implications for adult humans provided with a cochlear implant after a long period of deafness. The cats in our DS group (neonatally deafened with minimal experience of ICES until chronic stimulation was initiated at eight months of age) correspond most closely to human implantees who are deafened early (prelingually) and receive a cochlear implant as adults. Compared with prelingually deaf child implantees, and postlingually deaf adult implantees, this patient group consistently derives the poorest benefits from their implants, but their performance improves over a year or more post-implantation (e.g., Teoh *et al.*, 2004; Santarelli *et al.*, 2008). The poor performance of these patients undoubtedly reflects a number of factors; among them are the probable occurrence of cross-modal plasticity in the AI (e.g. Kral & Sharma, 2012) and limited (or no) exposure to spoken language during the critical period for language acquisition (e.g. Kuhl, 2004; Kuhl *et al.*, 2005), but it is possible that one of the factors contributing to improvement over the post-implantation period is the restoration of cochleotopy.

Postlingually deaf implantees also show progressive improvement over the year or so following implantation (e.g. McKay, 2005; Fu & Galvin, 2007). Profound bilateral adult-onset deafness has been shown in both human (Moore *et al.*, 1997) and animal (Powell &

Erulkar, 1962) studies to result in neuronal atrophy in auditory brainstem nuclei, but there appear to have been no studies of the effects of such deafness on the functional organization of auditory cortical or subcortical structures. The improvement shown by postlingually deaf human implantees undoubtedly reflects plasticity in numerous hearing- and language-related brain areas, as implantees learn to process the input provided by the device, which is unnatural and impoverished compared to that experienced prior to deafness. However, the restoration of deafness-degraded aspects of auditory cortical function, indicated in our study and that of Vollmer and Beitel (2011), is also likely to be a contributing factor.

Evidence bearing on the effects of deafness and implant use on the organization of auditory cortex in human implantees is unfortunately limited. Guiraud et al. (2007) have presented compelling auditory evoked potential evidence of cochleotopic organization in auditory cortex of a group of adult implantees (heterogeneous with respect to etiology and duration of deafness) within 3-8 months of implantation. Although the evidence from our and the other studies cited above suggests that cortical cochleotopy would have been lost in the patients in their study with early onset deafness, there appears to be no evidence on cochleotopic organization in deaf humans prior to implantation. As Guiraud et al. (2007) suggest, it would be of great interest to conduct a longitudinal study from the time of implant switch-on to investigate plasticity of cochleotopic organization in deaf humans.

Some suggestive evidence is, however, provided by a recent psychophysical study of a single human patient in whom a multi-electrode mid-brain prosthesis was implanted along the putative (dorsolateral to ventromedial) axis of the inferior colliculus. Lim et al. (2013) reported that, after ~4 months of implant use, the ordering of pitch percepts associated with electrical stimulation at different dorsoventral points across the colliculus was in accordance with the collicular tonotopy seen in animals. This pitch ordering was not observed immediately after implant activation, suggesting that cochleotopy in one or more auditory system structures at the level of the midbrain or above associated with pitch perception had

been degraded by deafness but was restored by chronic stimulation. This observation therefore suggests that an important feature of the perceptual experience of implantees who receive their implant after an extended period of deafness (viz., ordering of pitch percepts) depends on plastic changes of the sort we have described in auditory cortex.

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Abbreviations

AAF	Anterior auditory field
ABR	Auditory brainstem response
AES	Anterior ectosylvian sulcus
AI	Primary auditory cortex
BE	Best electrode
CG	Common ground
DS	Delayed stimulation
MS	Minimal stimulation
EABR	Electrically evoked auditory brainstem response
ICES	Intra-cochlear electrical stimulation
MP	Monopolar
PES	Posterior ectosylvian sulcus
SEM	Standard error of the mean
SNHL	Sensori-neural hearing loss

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Table 1. Details of electrical stimulation regimes, age, and number of responsive recording sites for individual animals

Group	Animal ID	Chronic Stimulus Regime	Age (months)	Acute Stimulus	Recording Sites	Responsive Sites	Tuned Sites
Minimal Stimulation	986	None	10	MP	80	51	39
	989	None	10	MP	71	28	22
	994	None	9	MP	64	26	26
	996	None	9	MP	26	20	20
Delayed Stimulation	973	CG	15	CG	62	44	37
	975	CG	15	CG	75	38	29
	976	MP	14	MP	79	52	49
	978	MP	14	MP	72	26	25
	979	MP	14	MP	79	63	59
	980	CG	15	CG	75	59	53

Individual animals in the two experimental groups are specified by identification (Animal ID) number; age is that at time of acute experiment; number of recording sites; responsive sites is that at which responses to electrical stimulation were recorded; tuned sites is that at which a best electrode could be determined. CG, common ground; MP, monopolar.

Table 2. Characteristics of ICES-evoked responses in putative AI

Group	**Acute Stimulation**	**BE Threshold (dB re EABR threshold)**	**BE dynamic range (dB)**	**Tuned responsive recording sites (%)**	**Depth of tuning (dB)**
MS	MP	-0.6 ± 0.3	2.9 ± 0.3	84.0	1.2 ± 0.1
DS	MP	0.002 ± 0.1*	2.2 ± 0.2*	88.7	0.52 ± 0.05*
	CG	-2.7 ± 0.4	4.6 ± 0.3	87.0	2.4 ± 0.2

Values are means ± standard error of the mean. Details of measures given in text.

* Significant difference ($p < 0.05$) from corresponding MS group.

Figure Legends

Figure 1. A: Photograph of the right auditory cortex in a MS animal (cat 986) illustrating the recording sites (white crosses). B,C: Representative BE maps of putative AI in a MS animal (cat 986). D: Experimental time lines for the MS and DS groups. E,F: Representative BE maps of putative AI in a DS animal (cat 976). Filled symbols represent the best electrode at each recording site (gray indicates a 'broadly tuned' response, see text for details). Electrode 1 is most apical; electrode 8 most basal. Open symbols with cross indicate recording sites that were responsive to ICES but at which a BE could not be determined. Cross symbols indicate non-responsive recording sites. BEs within grid are those obtained from electrodes in the planar array. Solid gray line indicates recordings made down the rostral bank of PES (posterior ectosylvian sulcus). Vertical dashed regions in B and E and lines in C and F indicate "restricted region" of analysis (see text for details). SSS, suprasylvian sulcus; AES, anterior ectosylvian sulcus; DVCR indicator = 2 mm bar lengths (applies for both panels B and E); D, dorsal; V, ventral; C, caudal; R, rostral; CR Position: caudorostral position measured from the tip of the PES.

Figure 2. Cochleotopic organisation for all animals in the MS (left column) and DS (right column) groups (cat numbers top left of each panel, correlations for 'restricted-region' (top) and 'best-fit' (bottom) analyses at end of abscissae). Symbols represent the BE at each recording site (see text for details). As in Figure 1, vertical dashed lines indicate region for which 'restricted-region' analysis was carried out. Solid line indicates best (straight-line or 'broken stick') fit to all data points (see text for details). *: Significant BE – CR position correlations (p-value < 0.01, see text for details). Arrow indicates the location of the tip of the anterior ectosylvian sulcus; other conventions as in Figure 1.

Figure 3. (A) The mean (± SEM) cochlea-to-cortex mapping correlations for putative AI (Pearson correlations; see text for details) in the MS group (n= 4) and the DS group. Analysis is shown for both the "restricted-region" (open bars) and the "best-fit" (filled and striped bars) analyses (see text for details), with the same colours used for panels A, B and C. For the DS group, means are shown for all animals (n=6; black bar) and for the animals in which the majority of recordings were in putative AI (i.e. excluding 980; n=5; striped bar). (B) Mean (± SEM) cochlea-to-cortex mapping for putative AI for the

DS group (n = 3); mapping ratios cannot be calculated for the MS group, as no members of that group had a significant BE – caudorostral position correlation. (C) Mean activated area at 2 dB above minimal cortical threshold for the two groups.