Research Article

Neural ITD Sensitivity and Temporal Coding with Cochlear Implants in an Animal Model of Early-Onset Deafness

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ABSTRACT

Users of cochlear implant (CI) face challenges in everyday situations such as understanding conversations in noise, even with CIs in both ears. These challenges are related to difficulties with tasks that require fine temporal processing such as discrimination of pulse rates or interaural time differences (ITD), a major cue for sound localization. The degradation in temporal processing and ITD sensitivity are especially acute in those who lost hearing in early childhood. Here, we characterized temporal coding and ITD sensitivity of single neurons in a novel animal model of early-onset deafness. Rabbits were deafened as neonates and deprived of auditory stimulation until they reached adult age when single-unit recordings from the auditory midbrain were made chronically using an unanesthetized preparation. The results are compared to measurements from adult-deafened rabbits with normal auditory development to understand the effect of early-onset deafness on neural temporal coding and ITD sensitivity. Neurons in the inferior colliculus (IC) of early-deafened rabbits were less likely to show sustained, excitatory responses to pulse train stimulation and more likely to show suppressive responses compared to neurons in adult-deaf animals. Fewer neurons showed synchronized responses to pulse trains at any rate in the early-deaf group. In addition, fewer neurons showed significant ITD sensitivity in their

overall firing rate in the early-deaf group compared to adult-deaf animals. Neural ITD discrimination thresholds in the early-deaf group were poorer than thresholds in adult-deaf group, especially at high pulse rates. The overall degradation in neural ITD sensitivity is consistent with the difficulties encountered by human CI users with early-onset hearing loss. These results lay the groundwork for investigating whether the degradations in temporal coding and ITD sensitivity observed in early-deaf animals can be reversed by appropriate CI stimulation during development.

Keywords: cochlear implant, inferior colliculus, interaural time difference, temporal coding, binaural hearing, early-onset deafness, auditory deprivation

INTRODUCTION

Cochlear implants (CI) are highly successful neural prosthetic devices that provide a sense of hearing for those with severe to profound hearing loss. Speech reception with CIs in good performing users is near normal in quiet environments. However, CI users still face difficulty understanding speech in everyday noisy environments and there is a large variability in performance among CI users. Most CI users also have difficulty with pitch perception and sound localization (Grantham et al. 2007; Wilson and Dorman 2008), even if they are implanted on both sides. These

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difficulties are particularly acute for those with early onset of deafness who experienced a long period of auditory deprivation (Lazard et al. 2012).

Some of these difficulties may stem from limitations in temporal processing of electrical stimulation. In most CI listeners, the ability to discriminate the rates of electric pulse trains is limited to ~300 pulses per second (pps) (Townshend et al. 1987; Zeng 2002; Kong et al. 2009). Rate discrimination performance is even poorer in subjects with early-onset hearing loss (Tong et al. 1988; Busby et al. 1993; Cosentino et al. 2016). A limitation in temporal coding of high-rate electric pulse trains has also been observed in the auditory midbrain of anesthetized, adult-deafened animals (Snyder et al. 1995; Hancock et al. 2012). Temporal coding in the auditory midbrain and cortex is further degraded in animals that were neonatally deafened or congenitally deaf (Vollmer et al. 2005; Beitel et al. 2011; Hancock et al. 2013).

The difficulties CI listeners experience with sound localization, even with implants on both sides (bilateral CIs), is likely related to their poor sensitivity to binaural cues. Specifically, bilateral CI users' sensitivity to interaural time differences (ITDs) is much poorer than that of normal-hearing listeners and highly dependent on stimulus parameters such as intensity and pulse rate. In particular, CI listeners' perceptual sensitivity to ITDs degrades markedly with increasing pulse rate (Kan and Litovsky 2015; Laback et al. 2015). ITD discrimination is even worse in those who lost hearing early in life and received implants as adults (Litovsky et al. 2010). Neural ITD sensitivity to bilateral CI stimulation measured in the auditory midbrain of anesthetized, acutely deafened animals also degrades rapidly with increasing pulse rate (Smith and Delgutte 2007; Hancock et al. 2012), although ITD sensitivity with low-rate electrical stimulation can be as sharp as with acoustic stimulation in animals with intact cochleas (Vollmer 2018). Neural ITD sensitivity in the auditory midbrain and cortex of congenitally deaf cats is further degraded compared to sensitivity in adult-deafened cats in anesthetized preparations (Hancock et al. 2010; Tillein et al. 2010).

Few studies have investigated temporal coding and ITD sensitivity with CI stimulation in unanesthetized preparations (Chung et al. 2014, 2016; Johnson et al. 2016), and these studies have only been performed in animals with normal auditory development that were deafened as adults. The upper limit of temporal coding of electrical stimuli in auditory midbrain neurons is substantially underestimated under anesthesia (Chung et al. 2014). Anesthesia also alters the spontaneous firing rate and temporal response patterns to periodic pulse trains in the inferior colliculus (IC). The pulse rate dependence of neural ITD sensitivity measured in unanesthetized animals better

matches human perceptual performance than observations from anesthetized animals (Chung et al. 2016).

The goal of this study was to characterize temporal coding and ITD sensitivity of IC neurons with bilateral CIs in a novel unanesthetized rabbit model of neonatal deafness. The results are compared to earlier data from bilaterally implanted rabbits with adult-onset deafness (Chung et al. 2016). In particular, ITD sensitivity was measured over a wide range of pulse rates to understand the interactions between temporal coding, ITD sensitivity, and age at onset of deafness.

MATERIALS AND METHODS

Animals

Three Dutch-belted rabbits (two females and one male) were deafened as neonates and bilaterally implanted at 2–3 months of age. Single-unit recordings from the inferior colliculus commenced at adult age (6 months) and lasted for 6 months (Table 1). All procedures were approved by the animal care and use committee of Massachusetts Eye and Ear.

Neonatal Deafening

The three rabbits were deafened as neonates by daily injection of neomycin (60 mg/kg/day, S.C.), adapting methods used in cats (Snyder et al. 1990; Vollmer et al. 1999; Fallon et al. 2009) and ferrets (Hartley et al. 2010). Auditory brainstem response (ABR) thresholds to acoustic clicks measured at P16 under sedation (midazolam 2 mg/kg I.M., fentanyl 10 mcg/kg I.M.) revealed ~50 dB elevation in thresholds compared to age-matched normal animals from the same litter. ABR thresholds measured at P21 after five more days of injections confirmed the absence of ABR to acoustic clicks up to ~110 dB SPL.

Surgical Procedures

At 2–3 months of age, the rabbits underwent a surgery to implant intracochlear electrode arrays bilaterally. The surgical procedure was the same as described in previous reports for adult animals (Chung et al. 2014, 2016). Briefly, the procedure was performed under anesthesia (xylazine 6 mg/kg S.C., ketamine 35 mg/kg I.M. then maintained by isoflurane 2.5 % delivered via facemask in O₂ 0.8 l/min) and strict aseptic condition. In each ear, a bullectomy was made to visualize the round window. The round window was enlarged with a bone drill to facilitate the insertion of an HL-8 eight contact cochlear implant array (Cochlear Ltd.). The round window was sealed with crushed muscle. CI

TABLE 1

Summary of deafness, recording history, and histological processing for each animal

Rabbit ID	Sex	Onset of deafness	Age in days at implantation	Age in days at habituation/recording sessions	Number of habituation/recording sessions	Number of units recorded	Age in days at perfusion	Side	Estimated number of total SGNs
I1	F	Neonatal	51	216–367	34	73	579	R ^a	14,437
								L ^a	13,850
I4	F	Neonatal	52	218–307	18	26	333	R ^b	22,543
								L ^b	19,886
I5	M	Neonatal	94	223–363	26	27	419	R ^b	11,211
								L ^b	16,225
A62	F	Adult	248	268–425	38	29	594 ^c	N/A	N/A
A71	F	Adult	352	363–534	52	44	N/A	N/A	N/A
B05	F	Adult	256	272–429	71	67	794 ^c	N/A	N/A
B07	F	Adult	309	323–613	53	46	1882 ^c	N/A	N/A
B63 ^d	F	Adult	434	N/A	N/A		519	R ^b	21,243
								L ^b	17,401
Normal 1	F	N/A	N/A	N/A	N/A	N/A	599	R ^b	25,572
								L ^b	29,981
Normal 2	F	N/A	N/A	N/A	N/A	N/A	486	R ^a	24,081
								L ^a	23,623

For adult-deafened rabbits, the onset of deafness is the same as the age at implantation (i.e., all adult-deafened rabbits were deafened during the CI surgery)

^aEmbedded in araldite and stained with toluidine blue

^bEmbedded in celloidin and stained with H&E

^cOnly the brain tissue was processed

^dNo data included in the current study was collected from this rabbit

connectors were sealed with silicon and sutured under the skin.

At 6 months of age, a second surgery was performed to attach a headpost and expose the CI connectors. A brass bar and a stainless steel cylinder were attached on the skull with stainless steel screws and dental acrylic. Silicon seals were removed from the CI connectors, which were attached to the cylinder for stimulation during neurophysiological recording sessions. After recovery from headpost surgery (~1 week), rabbits were habituated to the experimental setup until they could sit quietly for 2–3 h with the head fixed while receiving electrical stimulation. After the habituation period (1–2 weeks), a third surgery was performed to enable access to the IC via a craniotomy. The exposed area was covered with topical antibiotic ointment and sealed with dental impression material. Single-unit recording sessions started after 2–3 days of recovery.

Electrophysiological Methods

Single-Unit Recordings. Four-contact polyimide-insulated platinum/iridium linear microelectrode arrays (150 μ m spacing between contacts, 12.5 μ m site diameter; MicroProbes) were used to record single-unit activity from the IC. Recording electrodes

were advanced in a dorsoventral direction through the occipital cortex down to the IC, which was identified by the entrained multi-unit activity to the search stimuli. We sampled neurons across all depths where this background entrainment was observed. The point of entry for the penetration was systematically varied within the craniotomy and the craniotomy was extended over the duration of the experiment as to widely sample the IC. In addition, in most animals, we recorded from both ICs.

To reduce the electrical stimulus artifact, recordings were made differentially between one contact showing clear spike activity and a local reference obtained by averaging the signals from the three remaining contacts. Signals from the recording electrodes were acquired by a unity gain headstage (HST/16050; Plexon) and then filtered (100–8000 Hz) and amplified (PBX2; Plexon). The conditioned signals were sampled at 100 kHz using a 12-bit A/D converter.

Two methods were used for further reducing the stimulus artifact. For low pulse rates (<200 pps), the stimulus artifact was removed by a gate-and-interpolate technique (Heffer and Fallon 2008). For measurements of ITD sensitivity at higher pulse rates, an offline artifact rejection method based on template subtraction was used (Buechel et al. 2018). The

artifact template was calculated by averaging the raw waveforms from the recording electrode across all trials for each stimulus. Subtracting the average template from the raw waveform of each trial allowed detection of spikes that temporally overlap with the artifact.

Stimuli. Electric stimuli were the same as in the previous study in adult-deafened animals used for comparison (Chung et al. 2016). All stimuli were trains of biphasic pulses (50 μ s/phase) delivered to each cochlear implant through a pair of custom-built, high-bandwidth, isolated current sources. Stimulation was between the most apical and most basal intracochlear electrodes, which excites neurons over a wide range of the tonotopic axis. The stimulus used for searching for single units was a sequence of three pulses presented successively to both ears (diotically), the left ear, and the right ear with a 100-ms interval between each pulse and a 200-ms silent interval between the triplets.

Upon isolating a single unit, the response to the search stimulus was obtained as a function of current level to determine the response threshold. Spontaneous firing rate was measured by recording spikes without any stimulation for 30 s. All units showing a driven response to the search stimuli, whether excitatory or inhibitory, were further studied and included in the database.

Next, responses to diotic pulse trains (300 ms, repetition period 600 ms) were measured for pulse rates ranging from 20 to 1280 pps in approximate half-octave steps (excluding 28 Hz), with 12 repetitions to assess the temporal response pattern. Stimuli were usually presented at 1–6 dB above the threshold of synchronized responses to a single diotic pulse.

ITD sensitivity was characterized by acquiring responses while varying the relative timing of pulse trains delivered to the two implants with zero interaural level difference (ILD). ITD sensitivity to electrical stimulation is highly sensitive to the current level (Smith and Delgutte 2007). We first varied the ITD from -1500 to $+1500$ μ s in 300 μ s steps at 80 pps for three to four current levels within the dynamic range determined from the response to search stimuli. The stimulus level yielding the strongest ITD sensitivity was selected for detailed measurements. ITD was then varied from -2000 to $+2000$ μ s in 200 μ s steps with 10 repetitions at each ITD. To investigate the effect of pulse rate on ITD sensitivity, pulse rates ranging from 20 to 640 pps in one octave steps were randomly interleaved with the ITD values tested, resulting in a matrix of firing rates. (In early experiments in some adult animals, ITD sensitivity was not always measured for every pulse rate. (Chung et al. 2016)). When time permitted, a few more current levels were tested.

Experimental Design and Statistical Analysis

Neural data from the three early-deafened rabbits are compared with reanalyzed data from four adult-deafened rabbits used in a previous study (Chung et al. 2016). These rabbits were deafened and implanted at 8–10 months of age and studied electrophysiologically for 6 months. Deafening in these rabbits was performed at the time of cochlear implantation via injection of distilled water into the cochlea that results in hair cell death through osmotic stress (Chung et al. 2014). Aside from the timing of deafening, cochlear implantation, and neural recordings, all procedures in the adult-deaf rabbits were essentially the same as in the early-deaf rabbits. Detailed history of age at implantation and recording sessions for both groups of rabbits is given in Table 1. **Excitatory and Suppressive Responses.** In unanesthetized animals, electric pulse train stimuli can produce either increases or decreases in IC neural activity over the background activity (Chung et al. 2014). In order to test whether the prevalence of excitatory and suppressive responses is altered in early deafness, the “sustained” firing rate during pulse train presentation (“on-period”) was compared to the sustained firing rate during the silent interval between stimuli (“off-period.”) The sustained on-period response was isolated from the preceding onset response by excluding the first 30 ms of the 300-ms pulse train duration from analysis. Similarly, the sustained off-period response was isolated from the preceding “rebound” response by excluding the first 100 ms of the 300-ms interstimulus silent interval. This isolation of sustained responses was done for analysis of responses to diotic pulse trains as a function of pulse rate but not for characterizing ITD sensitivity.

To quantify the relative prevalence of excitatory and suppressive responses for each pulse rate, the on-period and off-period firing rates were compared on a trial-by-trial basis using a paired *t* test. If the mean firing rate during the on-period was significantly higher (resp. lower) than the mean firing rate during the off-period ($p < 0.01$, two-tailed), the response was considered excitatory (resp. suppressive). We further defined an “*E/S* index” to quantify the relative prevalence of excitatory and suppressive responses across pulse rates for each unit. Specifically, the excitatory area *E* (orange shading in Fig. 2a, c) was defined as the sum of the “on-period” firing rates minus the “off-period” firing rates over pulse rates that evoked a significant excitatory response. Similarly, a suppressive area *S* (green shading in Fig. 2b, c) was defined by summing firing rates across pulse rates that evoked a suppressive response. The *E/S* index is the ratio $(E - S)/(E + S)$ and ranges from -1 for purely suppressive responses to $+1$ for purely excitatory responses.

Temporal Coding. To quantify the degree of synchrony of spikes to the electrical pulses and determine the upper frequency limit of synchrony for each unit, the stimulus pulse train was cross-correlated with the spike trains (Hancock et al. 2012; Chung et al. 2014). The 30-ms onset response was excluded from the cross-correlation analysis to avoid biasing the synchrony estimate by a single prominent peak. To assess the statistical significance of cross-correlation peaks, the cross-correlation was computed for 5000 random spike trains containing same number of spikes as the original neural recording. A confidence bound for the null hypothesis of no correlation was defined as the 99.5th percentile of the cross-correlograms across the 5000 random spike trains. A peak in the cross-correlogram was considered significant when it exceeded the confidence bound. The cross-correlogram peak height normalized by the 99.5 % confidence bound was used as a measure of the degree of synchrony for each pulse rate. In addition, the upper rate limit of synchronized responses was computed by linearly interpolating the height of the cross-correlogram peaks to find the pulse rate where the peak height intercepted the confidence bound.

Neural ITD Sensitivity. ITD tuning curves were obtained by averaging the spike counts across all stimulus trials and over the entire stimulus duration (0–300 ms), including the onset response. Onset responses were included for the analysis of ITD sensitivity because they contain additional information about ITD over sustained responses that can be important perceptually (Smith and Delgutte 2007). Neural sensitivity to ITD was quantified by two metrics derived from the ITD tuning curves: the ITD signal-to-variance ratio (STVR) (Hancock et al. 2010) and the just-noticeable difference (JND) in ITD based on signal detection theory (Shackleton et al. 2003; Chung et al. 2016). ITD STVR is an analysis-of-variance-based metric that represents the fraction of variance in firing rates due to changes in ITD relative to the total variance in firing rate across both stimulus trials and ITDs. Responses were considered to be ITD sensitive when the STVR was significantly greater than zero (F test, $p < 0.01$). The degrees of freedom for the F test were (20,189) for most cases when 21 ITD values were tested over 10 repetitions.

To obtain the neural ITD JND, ITD tuning curves were first fit with a cubic spline to smooth out noise that might result in artifactually small JNDs. Both the mean and the variance of the spike count were smoothed. Then, a modified standard separation, D , from the reference ITD was calculated from the spline curves as follows (Simpson and Fitter 1973; Hancock and Delgutte 2004):

$$D_{\text{ITD}, \text{ITD}+\Delta\text{ITD}} = \frac{|\mu_{\text{ITD}} - \mu_{\text{ITD}+\Delta\text{ITD}}|}{\sqrt{(\sigma_{\text{ITD}}^2 + \sigma_{\text{ITD}+\Delta\text{ITD}}^2)/2}}$$

where μ_{ITD} is the mean spike count at the reference ITD, $\mu_{\text{ITD}+\Delta\text{ITD}}$ is the spike count at the test ITD, and σ_{ITD} and $\sigma_{\text{ITD}+\Delta\text{ITD}}$ are their respective standard deviations. The JND is defined as the ΔITD when $D=1$. If $D < 1$ over the entire range of ITDs tested, the JND is undefined. For each ITD tuning curve, the reference JND was optimized by searching over all tested ITDs within the rabbit's ethologically relevant range (± 300 μs) (Day et al. 2012) to find the reference that yielded the smallest JND.

The shapes of ITD tuning curves were classified by fitting a template consisting of the sum of Gaussian and sigmoid functions (Chung et al. 2016):

$$\text{Rate}(\text{ITD}) = A 2^{-\left(\frac{\text{ITD}-B}{C/2}\right)^2} + \frac{D}{1 + 3^{-2\left(\frac{\text{ITD}-B}{C}\right)}} + E$$

A , D , and E are scaling factors, B represents the center of both the Gaussian and the sigmoid functions, and C determines the half-width of the Gaussian function and the half-rise of the sigmoid function. The fitted curves were classified into four types: monotonic, peak, trough, and other (unclassified). ITD curves that were poorly fit by the template ($r^2 < 0.75$) were left unclassified. The best ITD (ITD_{best}) and the ITD of maximum slope (ITD_{MS}) were calculated from the fitted template. Only ITD tuning curves in response to pulse rates below 160 pps were used in these calculations so as to avoid multiple cycles of periodicity within the measured ITD range of ± 2000 μs .

Statistical Methods for Group Analysis. To compare the fractions of neurons showing excitatory/suppressive responses, significant synchronized responses, and significant ITD sensitivity as a function of pulse rate between the two deafness groups, two-way analyses of variance (ANOVAs) were run on the arcsine-transformed fractions. For categorical data such as distributions of ITD tuning shapes, χ^2 tests were used (Tables 2 and 3). Non-parametric tests (Wilcoxon rank-sum test and Kolmogorov-Smirnov test) were used to compare the medians and distributions of E/S index, spontaneous firing rates, and upper limit of synchronization between the two deafness groups. Lastly, two-tailed t tests were used to compare the means of ITD_{best} and ITD_{MS} between two deafness groups.

Inter-Animal vs. Between-Group Differences. Because the number of animals in each deafness group was modest (three for early-deaf, four for adult-deaf), it is important to separate the effect of inter-animal

TABLE 2

Summary of synchronization limits by deafness groups

Group	Unsync	Median ^a (pps)	IQR ^a (pps)
Early-deaf	34 % (32/94)	202	134–275
Adult-deaf	17 % (22/132)	177	112–272
	$\chi^2(1) = 9.1155$	$W = 5675.5$	$D_{62,110} = 0.1806$
	$p = 0.0025^b$	$n_1 = 62, n_2 = 110$	$p = 0.1341^d$
		$p = 0.3196^c$	

^aAnalysis limited to synchronized units only^b χ^2 test^cWilcoxon rank-sum test^dKolmogorov–Smirnov test

variability from genuine between-group differences on neural metrics of temporal coding and ITD sensitivity. For this purpose, we included a random “animal” factor nested within the deafness group factor when performing ANOVAs on neural response properties such as the *E/S* index, the cross-correlation peak height, and the ITD STVR. For the STVR, which is bounded between 0 and 1, an arcsine transform was applied to the data so that their distribution would more closely approximate the normality assumption of ANOVA. For the same reason, a square root transformation was applied to the cross-correlation peak height.

Longitudinal Trends in Response Properties Against Duration of Deafness. Because the neural recording sessions took place over an approximately 6-month duration (Table 1), we tested for possible trends in response properties such as spontaneous firing rate, *E/S* index, and ITD STVR as a function of time since onset of deafness (Table 3). Analysis of covariance (ANCOVA) was used to compare different models, including separate trends for each animal, a common trend for all animals, or no trend (a constant value), and select the best-fitting model in the least-squares sense. Separate ANCOVAs were performed for early-deaf and adult-deaf animals. For these analyses, the dependent variable (STVR, spontaneous rate) was transformed in the same way as for ANOVA to better approximate the normality assumption.

Histological Processing

Temporal Bones. Both cochleas from six rabbits were harvested following intracardiac perfusion (Table 1): the three early-deafened rabbits providing the primary data, two normal-hearing controls, and one adult-deafened rabbit distinct from the four providing the comparison physiological data. This latter rabbit was deafened and implanted by the same methods as used for the four adult-deafened rabbits providing comparison data for the current report, but it was not used for physiological data collection.

The cochleas were immersed in 10 % formalin solution for 24 h then moved to 120 mM ethylenediaminetetraacetic acid (EDTA) and 1 % glutaraldehyde (pH = 7) for 6–8 weeks for decalcification. The temporal bones were rinsed in distilled water and sequentially dehydrated with 70, 95, and 100 % ethanol. In four of the six rabbits (two early-deafened, one adult-deafened, and one normal hearing), both ears were embedded in celloidin (parlodion strips) (Mallinckrodt Chemicals, Phillipsburg, NJ, USA). Both ears of the remaining two rabbits (one early-deafened and one normal hearing) were infiltrated with increasing concentrations of araldite, oriented in molds and embedded in 100 % araldite (Electron Microscopy Sciences, Hatfield, PA, USA).

Temporal bones were sectioned at a thickness of 20 μ m and every tenth section was stained as described below. Celloidin sections were stained with hematoxylin and eosin (H&E) then mounted on glass slides. Araldite sections were stained with 1:3 water diluted ETS (EpoxyTissue Stain, contains toluidine blue and basic fuchsin, Electron Microscopy Sciences, Hatfield, PA, USA) at 60 °C for ~ 72 h and then mounted on Superfrost Plus slides (Fisher Scientific).

Stereological analysis of spiral ganglion neurons (SGNs) was accomplished by two observers blinded to animals' age, history of surgeries, and procedures. The analysis was performed using a BioPrecision motorized microscope stage with multi-axis controller (Ludl Electronic Product, Hawthorne, NY, USA), Lumenera video camera, and a Nikon E600 microscope and was assisted by Stereo Investigator (SI) software (MicroBrightField Bioscience, Williston, VT, USA). SGN counts were quantified using the optical fractionator technique (West et al. 1991). Every tenth section was used for counting. A contour of Rosenthal's canal was drawn around the entire ganglion under low magnification ($\times 20$), then the sampling grid was placed over the region of interest and sampling

sites were selected randomly by the software. The counting sites were examined with a $\times 100$ oil objective lens. The nucleoli of SGNs were marked if they came into focus, but the nucleoli in focus within the top plane were excluded (Ishiyama et al. 2011). After systemic sampling of all stained sections, the total number of SGNs was calculated by the software.

Electrode Track Reconstruction. In two early-deaf rabbits, we stained the electrode with India ink and then made electrolytic lesions during the last recording session while the animal was under anesthesia (xylazine, 6 mg/kg, S.C.; ketamine, 44 mg/kg, I.M.). Electrolytic lesions were made by passing 10 μ A of current for 30–60 s to mark the borders of the region showing evoked activity with CI stimulation. The rabbit was then perfused intracardially using a 10 % formalin solution. The brain was immersed in fixative for 24 h and then transferred to 25 % sucrose solution for several days. The brain was embedded in optimal cutting temperature (OCT) compound and sagittal sections (80 μ m) were cut with a cryostat at -15°C . Sections were mounted on gel-subbed slides after dehydrating with ethanol bath of increasing concentration up to 100 % and cell bodies were stained with azure-thionin. Electrode traces reaching the central nucleus of the IC were identified as they passed through the occipital cortex and superior colliculus. All lesions were located in the central nucleus of the IC.

RESULTS

We recorded from 126 single units in the IC of three neonatally deafened, bilaterally implanted rabbits (two females, one male). All three rabbits were deafened as neonates and implanted at age 2–3 months. Neurophysiological recordings began after the rabbits reached adult age at ~ 6 months. The rabbits only received stimulation through the CIs during the neurophysiological recording sessions, which lasted 2 h per session. The results from early-deaf rabbits are compared to reanalyzed, previously reported data from 188 neurons in four adult-deafened, female rabbits (Chung et al. 2016). Detailed history of age at implantation and recording sessions for both groups of rabbits is given in Table 1.

Count of Spiral Ganglion Neurons

Figure 1 shows mid-modiolar sections from a normal-hearing rabbit (A–C), an adult-deafened rabbit (D–F), and an early-deafened rabbit (G–I). The organs of Corti in the deaf animals (E, H) show damage and degeneration compared to the normal-hearing ani-

mal (B). Importantly, degeneration of inner and outer hair cells is apparent in both the early-deafened rabbit that was deafened by systemic injections of neomycin and the adult-deafened rabbit that was deafened by local injection of distilled water. High magnification images of Rosenthal's canal (Fig. 1C, F, I) reveal a reduction in SGN density in the deaf animals. To quantify the loss of SGN in deaf animals, the SGNs were counted by the optical fractionator technique in both ears of all six rabbits (see “Histological Processing” for details). Total SGN counts in the early-deafened animals ranged from 42 to 84 % of the mean SGN counts from the two normal-hearing controls ($\mu = 25,814$, $\sigma = 2900$). SGN counts from both ears of the adult-deafened rabbit were in the same range as the counts from early-deaf rabbits (Table 1). The fraction of SGN cell loss in the early-deaf rabbits was also in line with that observed in cats that were neonatally deafened with neomycin injections (Leake et al. 1991, 1992, 1999). The similarity in SGN cell counts between early-deaf and adult-deaf rabbits suggests that any difference in physiological properties between the two groups of animals is not likely to be caused primarily by differences in SGN number.

Dependence of Excitatory and Suppressive Responses on Deafness Group and Pulse Rate

Responses to electric pulse trains were measured as a function of pulse rate to compare the relative prevalence of excitatory and suppressive responses in the two groups of animals (early-deaf vs. adult-deaf). In both animal groups, there was substantial variability in the relative prevalence of excitatory and suppressive responses. Figure 2 shows temporal response patterns and firing rates to pulse trains stimuli for two neurons from early-deaf rabbits and one neuron from an adult-deaf rabbit. The first neuron from an early-deaf rabbit (Fig. 2A) shows excitatory responses at all pulse rates, but the response only lasts for 50–60 ms after stimulus onset for rates above 160 pps. Suppressive responses could not be observed in this neuron due to the absence of spontaneous activity. Such a lack of spontaneous activity was unusual with our unanesthetized preparation for both groups of animals. In contrast, the other neuron from an early-deaf rabbit (Fig. 2B) shows strong background activity and clear suppressive responses at all but the lowest pulse rate. The neuron from an adult-deaf rabbit (Fig. 2C) shows sustained responses up to 112 pps and a short-latency onset response at higher pulse rates. For pulse rates above 112 pps, the firing rate during the stimulus is lower than the rate in the silent interval between stimuli. All three neurons showed a low-pass dependence of the average sustained firing rate

FIG. 1. Cochlear histology. Hematoxylin and eosin-stained cochleas of a rabbit with normal-hearing (**A–C**), an adult-deafened rabbit (**D–F**), and an early-deafened rabbit (**G–I**). Mid-modiolar sections from the left cochleas are shown for all three animals. **A, D, G** Low magnification micrograph ($\times 2$). **B, E, H** Higher magnification images ($\times 20$) from the middle turn (boxed area in the first column). The normal cochlea shows normal cytoarchitecture of the organ of Corti (**B**) with intact inner hair cells (IHC) and outer hair

cells (OHC) (arrows). In deafened animals (**E, H**), inner and outer hair cells are absent, yet the tunnel of Corti remains intact (arrowhead **E**). **C, F, I** High magnification images ($\times 100$) of Rosenthal's canal (boxed area in the second column). Darkly stained Schwann cell nuclei and satellite cells are seen scattered among the ovoid-shaped SGNs. SGN density is lower in cochleas from deafened animals compared to the normal cochlea

(excluding the onset response) on pulse rate, but the neuron from the adult-deaf rabbit showed a sharper drop in firing rate than the neurons from early-deaf rabbits.

For each unit and pulse rate, the firing rate during the stimulus (“on-period”) was compared to the firing rate during the silent interstimulus intervals (“off-period”) using a paired *t* test to determine whether the electrical stimulation evoked a significant excitatory or suppressive response. We found a general reduction in excitatory responses and an increase in suppressive responses in the early-deaf group compared to the adult-deaf group. Whether the response was excitatory or suppressive often depended on pulse rate as illustrated in Fig. 2C. Figure 3A shows the fraction of IC units showing excitatory and suppressive responses to pulse train stimuli as a function of pulse rate for the two deafness groups. Positive ordinates indicate the fraction of excitatory responses and negative ordinates the fraction of suppressive responses. Excitatory responses dominate in the adult-deaf group up to 112 pps, with more than 50 % of

units showing excitatory response and fewer than 20 % showing suppression. (The fractions of excitatory and suppressive responses at each pulse rate do not sum to 100 % because, if a neuron's on-period response does not differ significantly from the background firing rate, it is considered neither excitatory nor suppressive.) IC units from the early-deaf group also exhibit a dominance of excitatory responses up to 112 pps, but the fraction of excitatory responses is substantially lower compared to the adult-deaf group. For pulse rates above 112 pps, more than 30 % of the units from the adult-deaf group continue to display excitatory responses and ~20 % of units show suppressive responses. For the early-deaf group, the proportions of excitatory and suppressive responses are more balanced, ranging from 20 to 30 % for both. Overall, more units showed suppressive response in the early-deaf group compared to the adult-deaf group. A two-way ANOVA on the arcsine-transformed fraction of excitatory responses showed significant effects of both pulse rate ($F(11, 11) = 20.29$, $p < 0.0001$) and deafness group ($F(1, 11) = 45.98$,

FIG. 2. Temporal response patterns (top) and average sustained firing rates (bottom) to electrical pulse trains of varying stimulation rates for two neurons from early-deaf rabbits (**A**, **B**) and one neuron from an adult-deaf rabbit (**C**). Alternating colors in the top indicate blocks of stimulus trials at different pulse rates. The orange-filled area indicates the amount of excitatory activity (excitatory area) and the green-filled area the amount of suppressive activity (suppressive area)

relative to the off-period firing rate. The neuron in **A** showed purely excitatory responses at all pulse rates, while the response of the neuron in **B** was primarily suppressive. The neuron in **C** showed a mixture of excitation and suppression depending on pulse rate. The *E/S* index (defined in “[MATERIALS AND METHODS](#)”) quantifies the relative prevalence of excitatory and suppressive responses across pulse rates

$p < 0.0001$). A parallel ANOVA for suppressive responses also showed significant effects of pulse rate ($F(11, 11) = 11.57$, $p = 0.0002$) and deafness group ($F(1, 11) = 50.47$, $p < 0.0001$).

We defined an *E/S* index for each unit to characterize the overall prevalence of excitatory vs. suppressive responses across pulse rates (see “[MATERIALS AND METHODS](#)”). The *E/S* index ranges from -1 for purely suppressive responses to $+1$ for purely excitatory responses (at all pulse rates). Figure 3B compares the distributions of *E/S* indices between the two groups. In both groups, there was a large number of almost purely excitatory responses ($E/S > 0.8$), but the fraction was lower in early-deaf animals. In contrast, the fraction of units showing predominantly suppressive responses (E/S index < 0) was higher for the early-deaf group. The two deafness groups showed statistically significant differences with respect to both median *E/S* indices (ED = 0.59, AD = 0.94, Wilcoxon rank-sum test, $n_1 = 85$, $n_2 = 128$, $W = 14,840$, $p = 0.0081$) and the shapes of the *E/S* distributions (Kolmogorov–Smirnov test, $D_{85, 128} = 0.2556$, $p = 0.002$). Examination of the data from individual animals showed that the median *E/S* indices were consistently lower in all three early-deaf animals compared to the four adult-deaf animals, suggesting the effect of deafness group was very robust.

The difference in incidence of excitatory and suppressive responses between the two deafness groups might be caused by a difference in spontaneous activity, which must be present for detecting suppressive responses. Earlier studies using anesthetized preparations have reported increased spontaneous firing rates (SR) of IC neurons in both neonatally deafened cats (Shepherd et al. 1999) and congenitally deaf cats (Hancock et al. 2010) compared to adult-deafened cats. Figure 3C shows the SR distributions for our two deafness groups, as well as data from a previous study of the IC in three normal-hearing, unanesthetized rabbits (Devore and Delgutte 2010). The median spontaneous firing rates were similar in the three groups (ED = 16.7 spikes/s, AD = 15.1 spikes/s, NH = 12.6 spikes/s) and did not differ significantly by a Kruskal–Wallis test ($\chi^2(2, 440) = 2.73$, $p = 0.256$). Focusing on the two groups of deaf animals, the shapes of the SR distributions also failed to differ significantly (Kolmogorov–Smirnov test, $D_{126, 186} = 0.1278$, $p = 0.1596$). Examination of the data from individual animals showed that median SRs were consistently lower in adult-deaf animals than in early-deaf animals, with the exception of one adult-deaf animal (B05), for which the median SR lay within the range of the early-deaf group. Repeating the rank-sum test with the data

FIG. 3. A Fraction of IC units showing excitatory (positive ordinates) and suppressive (negative ordinates) responses to pulse train stimulation as a function of pulse rate in early-deaf and adult-deaf rabbits. B Distribution of E/S index for the two groups. More

units show excitatory response (E/S index > 0) in adult-deaf group. C Comparison of spontaneous firing rate distributions between the early-deaf, adult-deaf, and normal-hearing animals

from B05 omitted revealed a significant effect of deafness group on the median SR ($n_1 = 126$, $n_2 = 119$, $W = 13,178$, $p = 0.0085$). Thus, the effect of deafness group on spontaneous activity, if any, was not very robust as it depended on the exclusion of one outlying animal. In contrast, the finding of a lower E/S index in early-deaf animals was very robust and highly significant whether B05 was included or not, suggesting the difference in incidence of excitatory and suppressive response between the two deafness groups is not dependent on a difference in spontaneous activity.

Dependence of Synchronized Activity on Deafness Group

For each neuron and pulse rate, the spike train was cross-correlated with the pulse train stimulus to characterize neural synchrony to the stimulus and define the upper frequency limit of synchrony (Hancock et al. 2012; Chung et al. 2014). Figure 4A, B shows cross-correlograms for each pulse rate for the same two units as in subpanels A and C of Fig. 2, respectively. The unit from the adult-deaf rabbit (Fig. 4B) shows short-latency (6–7 ms) correlation peaks (filled green) that exceed the 99.5 % confidence bound (gray shading) for pulse rates up to 160 pps. In contrast, for the neuron from an early-deaf unit (Fig. 4A), a significant peak is found only for the lowest pulse rate tested (20 pps) and this peak occurs at a longer latency (~13 ms). The upper limit of synchronization is defined as the pulse rate where the cross-correlogram peak height intercepts the 99.5 % confidence bound for a random spike train (Fig. 4C, D). The synchronization limit for the neuron from the early-deaf rabbit (40 pps) is lower than that from the adult-deaf rabbit (184 pps). (The apparent synchrony for pulse rates above 160 pps in Fig. 4B is an artifact of the gating technique used for stimulus

artifact cancellation and does not exceed the confidence bound. The gaps in the confidence bound represent segments that were gated out and therefore cannot contain spikes.)

Figure 5A compares the fraction of units that showed significant synchronized responses to the pulse train stimuli as a function of pulse rate for the two groups of animals. For nearly all pulse rates, more units had synchronized responses in the adult-deaf group compared to the early-deaf group, and the difference was most prominent for low pulse rates (< 112 pps). A two-way ANOVA on the arcsine-transformed fractions of synchronized responses showed significant effects of both pulse rate ($F(11, 11) = 24.19$, $p < 0.001$) and deafness group ($F(1, 11) = 11.5$, $p = 0.006$). Overall, more units from the early-deaf group (34 %) failed to show a synchronized response at any pulse rate tested compared to the adult-deaf group (17 %). This difference was statistically significant ($\chi^2(1, N = 226) = 9.1155$, $p = 0.0025$) (Table 2).

The height of the largest peak in the cross-correlogram, normalized by the 99.5 % confidence bound, was used as a measure of the strength of synchrony between the neural spike train and the stimulus pulse train. Figure 5B compares the normalized cross-correlogram peak heights for all neurons from the two deafness groups. For almost all pulse rates, the median peak heights were lower in early-deaf animals than in adult-deaf animals, indicating poorer synchrony. A three-way ANOVA was performed on the square root-transformed peak heights with pulse rate and deafness group as fixed factors and animal as a nested random factor. All three main effects were significant (pulse rate: $F(11, 2456) = 92.45$, $p < 0.0001$; deafness group: $F(1, 2456) = 10.05$, $p = 0.024$; animal: $F(5, 2456) = 8.96$, $p < 0.001$), as was the interaction between pulse rate and deafness group, reflecting a larger effect of group at low pulse

Fig. 4. **A, B** Cross-correlograms between stimulus pulse trains and neural spike trains for the same two neurons as in subpanels A and C of Fig. 2, respectively. Gray shading indicates the 99.5 % upper confidence bound for a random spike train; green-filled peaks indicate significance (correlation peaks exceeding the confidence bound). **C, D** Normalized height of the correlogram peak as a function of pulse rate. Peak heights are normalized to the 99.5 % confidence bound (dashed line). Green-filled points represent peak height exceeding the confidence bound. The pulse-locking limit (arrows) is defined as the rate where the peak height intercepts the 99.5 % upper confidence bound

rates ($F(11, 2456) = 2.43$, $p = 0.005$). Post hoc paired comparisons with Tukey-Cramer corrections suggested that the animal effect reflected significant differences in mean correlogram peak height among adult-deaf animals but not among early-deaf animals. The between-group variance in peak height (0.0095 square root units) was larger than the within-group, inter-animal variance (0.0028), suggesting that the finding of lower synchrony in the early-deaf group was robust to inter-animal variability.

Figure 5C compares the cumulative distributions of synchronization limits across neurons from the two deafness groups. Neurons that did not synchronize to the pulse train at any rate were excluded. The cumulative distributions from the two groups nearly overlap each other and do not statistically differ (Kolmogorov–Smirnov test, $D_{62, 110} = 0.1806$, $p = 0.1341$). Moreover, there was no significant difference in the median synchronization limits between the two groups (Wilcoxon rank-sum test, $n_1 = 62$, $n_2 = 110$, $W =$

5675.5, $p = 0.3196$) (Table 2). Thus, while the prevalence of synchronized responses and the strength of synchrony were decreased following neonatal deafness, the upper limit of temporal coding was not compromised in those neurons that did synchronize. This result is consistent with the observation that the differences in strength of synchrony between the two deafness groups are most prominent at low pulse rates, lower than the synchrony limits of many neurons.

ITD Sensitivity Depends on Age at Onset of Deafness

To characterize ITD sensitivity, we recorded responses of IC neurons to pulse trains with ITDs ranging from -2000 to $+2000$ μ s. Figure 6A shows the temporal response pattern to a 20-pps pulse train with varying ITD for the same neuron from an early-deaf rabbit as in Figs. 2A and 4A. The 20-pps pulse train generates synchronized responses, consistent with Fig. 2A, and additionally shows a modest preference for contralateral-leading (positive) ITDs. The firing rate vs. ITD curve (Fig. 6B) has a sigmoid shape rising towards contralateral-leading ITDs. Two metrics were used to quantify neural ITD sensitivity: the ITD STVR, an ANOVA-based metric that ranges from 0 (no sensitivity) to 1 (perfectly reliable tuning), and the JND in ITD based on signal detection theory. This neuron showed significant ITD sensitivity (STVR = 0.21, $F(20, 168) = 2.2998$, $p = 0.0022$) and an ITD JND of 830 μ s from a reference ITD of 210 μ s.

In adult-deaf animals, the ITD sensitivity of IC neurons tends to degrade with increasing pulse rate (Smith and Delgutte 2007; Hancock et al. 2012; Chung et al. 2016). We compared the dependence of ITD sensitivity on pulse rate for the two deaf groups. Importantly, every neuron that gave stable responses was tested for ITD sensitivity over a wide range of pulse rates (from 20 to 640 pps) even if it showed poor sensitivity at low pulse rates. Figure 7 shows ITD tuning curves measured for pulse rates from 20 to 640 pps in one unit from an early-deaf rabbit (same as in Figs. 2A and 6) and one unit from an adult-deaf rabbit. The unit from an early-deaf rabbit (Fig. 7A) shows a preference for contralateral-leading ITDs at very low pulse rates (≤ 40 pps) but little or no ITD tuning at higher rates (dashed lines in Fig. 7A). In contrast, the unit from an adult-deaf rabbit (Fig. 7B) shows sharper ITD tuning with increasing pulse rate up to 160 pps, beyond which responses are no longer ITD sensitive. The ITD STVR (Fig. 7C) was higher overall and statistically significant over a wider range of pulse rates (20–160 pps) in the adult-deaf neuron than in the early-deaf neuron (20–40 pps). The ITD JNDs (Fig. 7D) from the early-deaf

FIG. 5. **A** Fraction of units that showed significant synchronized response as a function of pulse rate. **B** Normalized cross-correlogram peak height as a function of pulse rate for all neurons from both deafness groups. The solid lines show the median peak height for

both groups. The ordinate reflects the square root transform applied to the data for ANOVA. **C** Cumulative distribution of upper limit of synchronized response for the two groups of rabbits. Units that did not show synchronized response at any pulse rate were excluded

neuron were higher (i.e., poorer performance) than the JNDs from the adult-deaf neuron and became unmeasurable (D did not reach unity within the range of ITDs tested) above 80 pps, while JNDs for the adult-deaf neurons improved with increasing pulse rate and were measurable up to 160 pps.

The contrast between the early-deaf and adult-deaf example neurons in Fig. 7 is representative of the corresponding neural populations. For all pulse rates tested, fewer neurons from the early-deaf group were sensitive to ITD, as measured by the STVR, compared to the adult-deaf group (Fig. 8A). This analysis was limited to units that were tested for at least two pulse rates. The fraction of IC neurons that showed significant ITD sensitivity at any pulse rate was lower in the early-deaf group (62 %) compared to the adult-deaf group (75 %), but this difference did not reach statistical significance ($\chi^2(1, N=154)=2.9500$, $p=0.086$). However, this test underestimates the effect of deafness group because it does not take into account the fact that the range of pulse rates over which neurons are ITD sensitive is wider in adult-deaf animals. Indeed, a two-way ANOVA on the arcsine-transformed fraction of ITD sensitive units showed

significant effects of both pulse rate ($F(5, 5)=15.67$, $p=0.0045$) and deafness group ($F(1, 5)=33.86$, $p=0.0021$). For the adult-deaf group, the highest fraction of ITD sensitive neurons occurred at 160 pps, where over 60 % were ITD sensitive. For the early-deaf group, the fraction of ITD sensitive units peaked at 80 pps.

Figure 8B shows that the mean ITD STVR was lower for the early-deaf group at all pulse rates tested with the largest difference above 80 pps. A three-way ANOVA on the arcsine-transformed STVRs showed significant effects of pulse rate ($F(5, 665)=13.37$, $p<0.0001$), deafness group ($F(1, 665)=7.41$, $p=0.038$), and the nested random factor “animal” ($F(5, 665)=3.43$, $p=0.0045$). Post hoc paired comparisons suggested that the animal effect was due to one adult-deaf animal (B07) that had a higher mean STVR than the other three animals in this group. When the three-way ANOVA was repeated with data from B07 omitted, the effect of deafness group remained significant ($F(1, 566)=7.30$, $p=0.039$), but the effect of animal was no longer significant ($F(4, 566)=1.87$, $p=0.114$). Thus, the finding of poorer ITD sensitivity in the early-deaf group is robust to inter-animal

FIG. 6. ITD sensitivity of the example neuron from Figs. 2A and 4A. **A** Temporal discharge patterns (dot rasters) as a function of ITD for a 20-pps pulse train. Alternating colors indicate blocks of stimulus trials at different ITDs. **B** Firing rate vs. ITD curve

FIG. 7. **A** Firing rate vs. ITD curves for different pulse rates from the same unit from the early-deaf rabbit as in Figs. 2A, 4A, and 5. Solid line indicates statistically significant ITD sensitivity ($p < 0.01$). **B** Firing rate vs. ITD curves for different pulse rates from a unit from an adult-deaf rabbit. **C** ITD STVR as a function of pulse rate for the two

example neurons. Filled dots represent statistical significant STVRs ($p < 0.01$). **D** ITD JND as a function of pulse rate for the two example neurons. ITD JNDs were unmeasurable (UM) for pulse rates above 80 pps for the early-deaf neuron and 160 pps for the adult-deaf neuron

variability and not dependent on a single adult-deaf animal with higher STVRs.

Finally, Fig. 8C compares the neural ITD JNDs from the two groups of rabbits as a function of pulse rate. Median JNDs (solid lines) were larger in early-deaf rabbits than in adult-deaf rabbits for all rates tested. (Unmeasurable JNDs were included in the median computations.) ITD JNDs were unmeasurable for more than half of the neurons at 20, 320, and 640 pps in early-deaf rabbits but only at 640 pps in adult-deaf rabbits.

Because ITD sensitivity requires synchrony to the stimulus in the inputs to the primary site of binaural interactions, we compared the fraction of IC neurons that showed significant ITD sensitivity at any pulse rate between neurons that did and did not synchronize to the pulse train stimulus. A higher fraction of synchronized units (71 %) was sensitive to ITD compared to non-synchronized units (48 %), and the difference was significant ($\chi^2(1, N=128) = 4.7675$, $p = 0.029$). This result suggests that the decreased prevalence of ITD sensitivity in the early-deaf group may be related to the decrease in synchronized responses in this group. However, in the early-deaf group, there was no significant correlation between

the upper limit of synchronization and the maximum STVR measured in each neuron (Kendall's $\tau = 0.14$, $p = 0.16$), suggesting that other factors than synchrony also influence ITD sensitivity. One such factor could be the balance between excitatory and suppressive responses, which clearly differed between the two animal groups. Although more neurons in all deafened animals with primarily excitatory responses (E/S index > 0) were ITD sensitive (72 %) compared to units with predominantly suppressive responses (55 %), this difference did not reach statistical significance ($\chi^2(1, N=128) = 3.4857$, $p = 0.0619$). Overall, ITD sensitivity was not strongly related to other response properties, the only robust effect being the presence of synchronized firings.

Shapes of ITD Tuning Curves and Tuning Metrics

The ITD tuning curves were fit to a flexible template to classify their shapes into four categories (see “[MATERIALS AND METHODS](#)”): monotonic, peak, trough, and other (unclassified). Only units that showed significant ITD sensitivity based on the STVR for at least one pulse rate below 160 pps were used for

Fig. 8. **A** Fraction of ITD-sensitive neurons as a function of pulse rate for early-deaf and adult-deaf animals. **B** Mean ITD STVR vs. pulse rate for early-deaf and adult-deaf animals. Error bars represent ± 2 S.E., which corresponds to a 95 % confidence interval for normal distributions. **C** ITD JNDs vs. pulse rate from individual neurons for early-deaf and adult-deaf animals. Solid lines represent the median JNDs. ITD JNDs were unmeasurable for more than half of the units at 20, 320, and 640 pps for the early-deaf group and at 640 pps for the adult-deaf group. The area of the circles at the top is proportional to the number of unmeasurable JNDs

classification. When significant ITD sensitivity was observed at multiple pulse rates, the ITD tuning curve with the highest STVR was used for shape classification. The relative incidences of the four shapes of ITD tuning are compared in Fig. 9A for the two deafness groups. There were slightly more “monotonic” shapes and fewer “peak” shapes in the early-deaf group compared to the adult-deaf group. However, the relative incidences of “trough” and “unclassified” types were similar in the two groups. Overall, the relative incidences of ITD tuning shapes did not

significantly differ between the two groups ($\chi^2(3) = 1.7166$, $p = 0.63$).

To further compare quantitative characteristics of ITD tuning between the two deafness groups, two ITD tuning metrics were calculated from the fitted curve: the best ITD (ITD_{best}) and the ITD of maximum slope (ITD_{MS}), where sensitivity of the mean firing rate to changes in ITD is maximal. ITD_{best} was only estimated from the peak-shaped ITD tuning curves. The distribution of ITD_{best} (Fig. 9B) in the early-deaf group showed a slight contralateral bias; however, this trend did not meet statistical significance (mean = +165 μ s, two-tailed t test, $t(23) = 1.9749$, $p = 0.0604$). In contrast, ITD_{best} in the adult-deaf group did show a statistically significant contralateral bias (mean = +199 μ s, two-tailed t test, $t(45) = 3.1763$, $p = 0.0027$), with 70 % of $ITD_{best} > 0$. There was no significant difference in mean ITD_{best} between the two deafness groups ($p = 0.7460$, $t(68) = -0.3252$, two-tailed t test). ITD_{MS} was calculated for all ITD tuning shapes except unclassified. ITD_{MS} was broadly distributed (Fig. 9C), with a mean near zero for both groups (ED – 21 μ s, AD – 95 μ s) and there was no statistical difference between the two means (two-tailed t test, $t(159) = -0.5979$, $p = 0.5507$). Thus, neither the distributions of ITD tuning shapes nor the ITD tuning metrics differed between the two groups; the only difference was that a significant contralateral bias in the ITD_{best} distribution was only observed in adult-deaf animals.

Lack of Longitudinal Effect on Temporal Coding and ITD Sensitivity

As shown in Table 1, the neurophysiological recordings from early-deaf rabbits were made from 7 to 12 months of age. The rabbits only received bilateral electrical stimulation during the neurophysiological sessions. The stimulation during these sessions might restart the experience-dependent maturation of temporal and binaural processing mechanisms interrupted by 6 months of auditory deprivation prior to the recording sessions. On the other hand, the amount of stimulation during the recording sessions might not be sufficient to prevent a continued degeneration of the auditory processing mechanisms. To distinguish between these two hypotheses, we tested for longitudinal effects on temporal coding and ITD sensitivity over the period of recording sessions in the early-deaf rabbits (Table 3). We used ANCOVA to test whether the three early-deaf animals shared a common trend or showed different trends (see “**MATERIALS AND METHODS**”). For both spontaneous firing rate and upper limit of synchrony, the ANCOVAs indicated it was appropriate to combine data from the three animals and revealed a significantly increasing trend in the combined data (SR: $F(1, 124) = 7.33$, $p = 0.0078$; synchrony limit: $F(1,$

FIG. 9. **A** Distribution of ITD tuning shapes for the two deafness groups. Analysis was limited to units with statistically significant ITDs based on ITD STVR. **B** Distribution of best ITDs of peak-shaped units. **C** Distribution of ITD_{MS}. Error bars above each histogram represent the means $\pm$ 1 S.D.

55) = 5.82, $p = 0.019$). In contrast, for the maximum ITD STVR and the E/S index, the ANCOVAs indicated it was more appropriate to fit separate trends for each animal, and none of the trends from individual animals were significant (Table 3). Thus, we did not find a consistent effect of duration of auditory experience on either excitability or ITD sensitivity in the early-deaf rabbits but found modest increasing trends for SR and synchrony limits when data were combined across animals.

A similar longitudinal analysis using ANCOVA was performed in three of the four adult-deaf rabbits; there were not enough data in the fourth rabbit (A62) to detect meaningful trends. Consistent with the results of Chung et al. (2016) obtained by a different method, no consistent effect of age was found for either spontaneous firing rates or ITD sensitivity (Table 3). Although significant trends for SR were found in two rabbits, the trends were in opposite directions in the two animals, so that there was no consistent pattern. We also failed to find any significant longitudinal trend for the synchronization limit

and the E/S index in the adult-deaf animals (Table 3). The lack of a trend for synchronization limits is consistent with an earlier study (Chung et al. 2014) in a different group of adult-deaf animals.

To summarize these longitudinal analyses, no consistent trend was found for either E/S index or ITD sensitivity in either deafness group, and there was also no trend for SR and synchrony limit in the adult-deaf animals. Although the modest increasing trend observed for synchrony limit in the early-deaf group could be interpreted as a beneficial effect of electric stimulation on temporal coding, the increase in SR in early-deaf animals is hard to interpret given the lack of robust differences in SR between the two deafness groups and between deaf and normal-hearing animals. Thus, the present results fail to give consistent evidence for a beneficial effect of cochlear implant stimulation during the neurophysiological recording sessions on response properties of IC neurons in the early-deaf group.

TABLE 3

Longitudinal trends in response properties in individual animals from both deafness groups				
Animal ID	Spontaneous rate	E/S index	Upper limit of synchrony	Max ITD STVR
I1 (ED)	$t = -2.71$, $p = 0.008$	$t = 1.74$, $p = 0.085$	$t = 2.41$, $p = 0.019$	$t = 0.64$, $p = 0.52$
I4 (ED)		$t = -1.28$, $p = 0.20$		$t = 0.93$, $p = 0.35$
I5 (ED)		$t = 0.17$, $p = 0.87$		$t = -1.65$, $p = 0.10$
A71 (AD)	$t = -2.21$, $p = 0.028$	$t = -1.04$, $p = 0.30$	No trend (best-fitting model is a constant)	$t = -1.28$, $p = 0.20$
B05 (AD)				$t = -1.69$, $p = 0.092$
B07 (AD)				$t = 2.96$, $p = 0.0035$

Entries show the results of t tests for the slopes of the best-fitting lines determined by ANCOVA. Where data from several animals appear in the same cell, the best-fitting ANCOVA model had a common trend for all animals. Data from adult-deaf animal A62 were not included because there were not enough data in this animal to detect meaningful trends. Bold text indicates statistical significance.

DISCUSSION

We characterized temporal coding and ITD sensitivity of IC neurons in neonatally deafened rabbits that received no stimulation until 6 months of age and compared the response properties to data from rabbits with normal auditory development that were deafened as adults. We found an overall reduction in the prevalence of excitatory responses relative to suppressive responses, as well as degradations in temporal coding and ITD sensitivity in the early-deaf animals in IC neurons that were driven by the electrical stimulation. The reduction in excitatory responses and loss of temporal coding was most pronounced at low pulse rates and was probably not due to a change in spontaneous activity since there were no robust differences in spontaneous firing rates between the two deafness groups. Fewer neurons were sensitive to ITD of periodic pulse trains in the early-deaf group for all pulse rates tested, with especially large differences at higher pulse rates. Neural ITD JNDs were also poorer in the early-deaf group compared to adult-deaf group.

Although the number of animals in each deafness group was modest (three early-deaf, four adult-deaf), the above results seem to be robust with respect to inter-animal variability. For the *E/S* index, the median indices in each of the early-deaf animals were lower than the median values in all of the adult-deaf animals, suggesting a very robust difference. Although there was significant inter-animal variability among the adult-deaf group (but not the early-deaf group) with respect to both ITD STVR and strength of synchronization, in both cases, the across-group variance was larger than the within-group variance, and the effect of early deafness on ITD STVR was still observed after data from one adult-deaf animal with higher STVRs were excluded. Spontaneous activity was the one exception to the robustness of the inter-group differences, as an effect of early deafness could only be detected by excluding data from one adult-deaf animal that appeared to be an outlier. Overall, inter-animal variability was larger in the adult-deaf group than in the early-deaf group. This may be because the adult-deaf experiments lasted over a longer period of time and their methods were not as fully standardized as in the early-deaf experiments (the main difference was that ITD sensitivity was not always measured for every pulse rate in some of the adult-deaf animals).

We cannot completely rule out that the observed differences in response properties between early-deaf and adult-deaf animals could be influenced not only by the age at onset of deafness but also by possible

differences in IC subregions sampled. However, this is unlikely given the strict procedures used to position the headbar and craniotomy reproducibly in each animal, the large number of electrode penetrations performed (Table 1), the deliberate efforts to sample from all the regions of the IC accessible through the craniotomy, and the fact that neurons were recorded from at all electrode depths over which electric stimuli evoked multi-unit activity (and therefore from a wide span of the cochleotopic axis of the IC).

The overall degradation in temporal coding observed in the current study is consistent with previous findings in anesthetized animals. The upper limit of synchronized responses of IC neurons is reduced in both neonatally deafened cats (Shepherd et al. 1999; Vollmer et al. 2005) and congenitally deaf cats (Hancock et al. 2013) compared to acutely deafened adult cats. However, the median synchronization limit in the IC in early-deaf animals in the present study (202 pps) is much higher than the temporal limits in the abovementioned studies in anesthetized animals (median 30–50 pps). In this respect, the effect of anesthesia on the limit of temporal coding is similar to that observed in adult-deaf animals (Chung et al. 2014).

The overall degradation in ITD sensitivity observed in the early-deaf group is consistent with previous studies of the IC (Hancock et al. 2010) and auditory cortex (Tillein et al. 2010) of anesthetized, congenitally deaf white cats that reported a reduced incidence of ITD sensitivity and poorer ITD sensitivity compared to acutely deafened cats with normal auditory development. However, in contrast to Hancock et al. (2010), we did not observe clear changes in the distributions of best ITDs or ITD of maximum slope.

Even in adult-deaf animals, ITD sensitivity with cochlear stimulation is poor compared to the sensitivity observed in the IC of normal-hearing rabbits with acoustic stimulation (e.g., Kuwada et al. 1987; Fitzpatrick et al. 2000; Devore and Delgutte 2010), although no detailed comparisons have been made for comparable stimuli. The comparatively weak ITD sensitivity in adult-deaf animals may seem discouraging from the perspective of improving binaural performance of CI users with early-onset deafness by providing binaural stimulation. However, we characterized ITD sensitivity for all neurons that provided stable recordings, whereas studies in normal-hearing animals often select for the presence of ITD sensitivity or for neurons tuned to low frequencies, which are more likely to be sensitive to ITD in the temporal fine structure. Moreover, we have shown previously that ITD sensitivity to low-rate electric pulse trains is often masked by unsynchronized background activity and

can be improved by selecting spikes synchronized to the stimulus (Chung et al. 2016). This spike selection process can be implemented by a biologically plausible coincidence detection mechanism (Buechel et al. 2018) and works in neurons from early-deaf animals as well as those from adult-deaf animals (not shown). The difference in ITD sensitivity between normal-hearing animals and deaf animals with cochlear implants becomes less severe when the differences in methods and stimuli are taken into account.

Lack of Increase in Spontaneous Activity in Early-Deaf Group

We did not find robust differences in spontaneous activity between early-deaf, adult-deaf, and normal-hearing animals. This lack of a robust effect contrasts with previous studies using anesthetized preparations, which reported higher spontaneous activity in both neonatally deafened (Shepherd et al. 1999) and congenitally deaf cats (Hancock et al. 2010, 2013) compared to acutely deafened cats with normal development. These studies did not report comparison data from normal-hearing animals. In normal-hearing animals, barbiturate anesthesia is known to cause a decrease in spontaneous activity in both the auditory midbrain (Bock and Webster 1974; Kuwada et al. 1989; Torterolo et al. 2002) and the auditory cortex (Zurita et al. 1994). Previously, we also found a clear decrease in spontaneous firing rates following injection of short-acting barbiturate in adult-deaf rabbits (Chung et al. 2014). It is possible that there is an interaction between anesthesia and the neural mechanism underlying the increase of spontaneous activity in early-deaf animals so that the increase is more prominent under barbiturate anesthesia.

Mechanisms for Effect of Early Deafness on IC Response Properties

Many studies have reported a change in the excitatory to inhibitory balance towards a reduction in inhibition in the central auditory pathway following various types and degrees of peripheral damage. At the synaptic level, downregulation of inhibitory synaptic gain has been observed in anatomical and brain slice studies following various types of hearing loss during development (for review, Takesian et al. 2009). Other studies in adult animals have shown restoration of sound-evoked responses following severe cochlear denervation (Chambers et al. 2016; Resnik and Polley 2017) or inner hair cell damage (Qiu et al. 2000; Salvi et al. 2016). This is interpreted as a form of homeostatic plasticity whereby the “central gain” is increased so as to maintain a constant activity level in the face of peripheral loss.

In contrast to these findings of increased central gain, we observed an increase in the incidence of suppressive responses and a reduction in excitatory responses in the IC in early-deaf animals. These effects could be mediated by a number of mechanisms, including increased inhibition, changes in intrinsic properties leading to an increase in threshold, or long-term depression of excitatory synapses, and any of these mechanisms could operate in the IC itself or at more peripheral stages in the brainstem. For example, Tirko and Ryugo (2012) reported both a reduction in the size of excitatory synaptic boutons and a reduction in the number of inhibitory synapses onto MSO principal cells in congenitally deaf cats compared to normal-hearing cats. Changes in both sodium and potassium conductances have been reported in the medial nucleus of the trapezoid body (MNTB) of congenitally deaf mice compared to wild-type mice (Leao et al. 2004; Leao et al. 2006). These changes could in turn alter binaural interactions in the medial and lateral superior olives (MSO and LSO) which MNTB neurons project to (Brand et al. 2002; Cant and Benson 2006).

One possible explanation for our failure to observe an increase in central gain is that the central auditory system responds differently to the partial peripheral hearing loss in adulthood produced in studies that observed central gain compensation compared to the profound neonatal deafening induced by ototoxic drugs used in the present study. Moreover, the studies reporting an increase in central gain typically used acoustic stimulation, while we used electric stimulation.

The degradations in temporal coding and ITD sensitivity observed in the IC of early-deaf animals might be caused by damage at any site along the ascending auditory pathway beginning in SGN. The demyelination of auditory nerve fibers and reduction in SGN counts observed in neonatally deafened cats (Hardie and Shepherd 1999) can contribute to the degradation in temporal coding (Resnick et al. 2018) and lead to inefficient binaural coincidence detection. However, demyelination and reduction in SGN density are not limited to early-onset deafness and have been also observed in cats deafened as adults (Shepherd and Javel 1997). Structural abnormalities have been observed in the endbulb synapses between auditory nerve fibers and spherical bushy cells of the cochlear nucleus (CN) in both congenitally deaf (Ryugo et al. 2005) and neonatally deafened cats (Ryugo et al. 2010). Because these synapses are specialized for transmission of precise temporal information to the targets of bushy cells in the medial superior olive (MSO), their disruption likely impairs ITD processing (O'Neil et al. 2011).

In short, degeneration in spiral ganglion neurons and cochlear nucleus synapses caused by ototoxic drugs likely has effect on temporal coding and binaural sensitivity; however, it is unclear whether early-onset deafness has more severe effect on these cells. The downregulation of synaptic inhibition in the brainstem or midbrain observed in many studies may not be sufficient to compensate for the effect of morphological abnormalities and loss of overall level of synaptic excitation and fully restore response to electrical stimulation following early-onset deafness. More physiological data from the ascending pathways to the midbrain in response to electrical stimulation (Clark 1969; Babalian et al. 2003) are needed to understand the mechanisms of degradation in temporal processing in early-onset deafness and suggest possible target sites for reorganization with electrical stimulation.

Implication for Human Performance and Rehabilitation

The present findings are in broad agreement with perceptual results from human CI listeners. Rate discrimination for electric pulse trains is generally poor and limited to below ~300 pps in most CI users and is even poorer in those with early onset of deafness (Tong et al. 1988; Busby et al. 1993; Cosentino et al. 2016). ITD sensitivity is limited to a similar range of pulse rates as rate discrimination (Ihlefeld et al. 2015) and is severely degraded in subjects with prelingual onset of hearing loss (Laback et al. 2015).

Chronic stimulation with CIs can partly reverse the degradations in the auditory pathway associated with long-term deafness. Specifically, chronic CI stimulation can help prevent the degeneration of SGN in cats deafened by ototoxic drugs (Leake et al. 1991, 1999). Chronic CI stimulation can also restore normal synaptic morphology in the CN (Ryugo et al. 2005; O'Neil et al. 2010) and MSO (Tirko and Ryugo 2012) of congenitally deaf cats. Finally, an enhancement in temporal coding following chronic CI stimulation has been observed in the IC of early-deafened cats in anesthetized preparations (Vollmer et al. 2005, 2017). It will be important to test whether such an improvement can also be observed in unanesthetized animals, where the synchronization limit is substantially higher (Chung et al. 2014). Testing whether ITD sensitivity can also be improved by appropriate bilateral CI stimulation during development in early-deaf animals would be valuable for understanding the developmental process of binaural circuitry and suggesting possible rehabilitation strategies that would improve binaural sensitivity in CI users with early onset of deafness.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest Y. Chung, B.D. Buechel, W. Sunwoo, J.D. Wagner, and B. Delgutte have no conflict of interest to declare.

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