Sensitivity to Interaural Time Differences in the Inferior Colliculus of Cochlear Implanted Rats With or Without Hearing Experience

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Declaration of Interests:

The authors declare that they have no competing interests.

Abstract:

For deaf patients cochlear implants (CIs) can restore substantial amounts of functional hearing. However, binaural hearing, and in particular, the perception of interaural time differences (ITDs) with current CIs has been found to be notoriously poor, especially in the event of early hearing loss. One popular hypothesis for these deficits posits that a lack of early binaural experience may be a principal cause of poor ITD perception in pre-lingually deaf CI patients. This is supported by previous electrophysiological studies done in neonatally deafened, bilateral CI-stimulated animals showing reduced ITD sensitivity. However, we have recently demonstrated that neonatally deafened CI rats can quickly learn to discriminate microsecond ITDs under optimized stimulation conditions which suggests that the inability of human CI users to make use of ITDs is not due to lack of binaural hearing experience during development. In the study presented here, we characterized ITD sensitivity and tuning of inferior colliculus neurons under bilateral CI stimulation of neonatally deafened and hearing experienced rats. The hearing experienced rats were not deafened prior to implantation. Both cohorts were implanted bilaterally between postnatal days 64-77 and recorded immediately following surgery. Both groups showed comparably large proportions

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of ITD sensitive multi-units in the inferior colliculus (Deaf: 84.8%, Hearing: 82.5 %), and the strength of ITD tuning, quantified as mutual information between response and stimulus ITD, was independent of hearing experience. However, the shapes of tuning curves differed substantially between both groups. We observed four main clusters of tuning curves – trough, contralateral, central, and ipsilateral tuning. Interestingly, over 90% of multi-units for hearing experienced rats showed predominantly contralateral tuning, whereas as many as 50% of multi-units in neonatally deafened rats were centrally tuned. However, when we computed neural d' scores to predict likely limits on performance in sound lateralization tasks, we did not find that these differences in tuning shapes predicted worse psychoacoustic performance for the neonatally deafened animals. We conclude that, at least in rats, substantial amounts of highly precise, "innate" ITD sensitivity can be found even after profound hearing loss throughout infancy. However, ITD tuning curve shapes appear to be strongly influenced by auditory experience although substantial lateralization encoding is present even in its absence.

Keywords:

cochlear implants, binaural hearing, interaural time differences, early onset deafness, electrophysiology, inferior colliculus

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1. Introduction:

Cochlear implants (CIs) have greatly improved the quality of life of more than half a million deaf patients, often restoring the ability to take part in spoken conversations. However, patients vary greatly in how much benefit their CIs give them. Here, hearing experience is an important factor, both prior to hearing loss as well as following implantation. One major challenge for CI patients is spatial hearing, and in particular, the use of interaural time differences (ITDs). In particular pre-lingually deafened subjects, even those who have received bilateral implants within the first 18 months of life, are usually unable to detect ITDs (Wickens 2002; van Hoesel 2004; Grieco-Calub and Litovsky 2010; Litovsky 2010; Litovsky et al. 2010; Litovsky 2011a; van Hoesel 2012; Kerber and Seeber 2012; Laback et al. 2015; Ehlers et al. 2017). In many cases, ITD thresholds of theses patients are, if at all measurable, orders of magnitude above their normal hearing peers who can resolve ITDs of a few tens of µs (Zwislocki and Feldman 1956). Post-lingually deafened CI users often perform significantly better than pre-lingually deafened peers, but even their thresholds are many times higher than those of their normal hearing experienced peers (Litovsky et al. 2010; Litovsky 2011b; Brughera et al. 2013). Furthermore, sound localization performance does not improve much with long-term CI exposure (Loizou et al. 2009; Chang et al. 2010; Litovsky 2011b; Kerber and Seeber 2012).

It is widely believed that lack of binaural exposure during an early “critical” period of the binaural auditory pathway development is a major factor contributing to the ITD insensitivity of human CI users. However, we recently demonstrated that neonatally deafened rats fitted with bilaterally synchronized CIs in young adulthood were capable of learning to lateralize ITDs with thresholds as low as 50 µs, comparable with their normal hearing peers (see Supplementary Fig. 1; Rosskothen-Kuhl et al. 2021). Thus, in spite of having no early hearing experience, these animals were able to make use of these cues that have been elusive to human CI listeners. This raises the possibility that reasons other than lack of early auditory experience may limit CI users’ ability to develop

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normal ITD sensitivity. These may include technology limitations of current clinical CIs such as the lack of synchronization between the left and right speech processors and the spread of electric fields resulting in blurring (Carlyon et al. 2007; Oxenham and Kreft 2014) across frequency bands. In addition, there may be prolonged periods without auditory input, either bilaterally or unilaterally, which has been shown to alter binaural processes under both acoustic and electric hearing (King et al. 1988; King et al. 2000; Gordon et al. 2014). However, the underlying mechanisms for these plastic changes, particularly for electric hearing, are not fully understood. Thus, a better understanding of how innate binaural processing mechanisms and experience dependent plasticity interact in a brain that receives stimulation only after prolonged early deafness could inform improved CI treatment strategies.

Several physiological studies have reported ITD sensitivity in the inferior colliculus under CI stimulation (Hancock et al. 2010; Hancock et al. 2012; Hancock et al. 2013; Chung et al. 2019). However, these studies only sparsely sampled ITDs within the respective animals' physiologically relevant range. In addition, ITDs well beyond the physiological range and therefore far wider than an animal would ever experience in nature were used. This greatly reduces the translatability of these studies in predicting behavioral performance limitations for physiologically relevant ITDs. Furthermore, earlier reports by Hancock et al. (2010); Hancock et al. (2012); Hancock et al. (2013) and Chung et al. (2019) excluded onset responses from the analysis, which is a surprising choice given that ITD perception is known to be heavily onset dominated (Brown and Stecker 2010; Stecker et al. 2013), and it has been demonstrated that the early response would encode the early stimulus (Heil 1998) which is often weighted the most heavily in perceptual lateralization judgments. We hypothesized that these methodological choices made in previous studies may have led to underestimates of "intrinsic" ITD sensitivity present in the auditory pathway under electric stimulation in the absence of early hearing experience. Thus, in this study we revisited the question of physiological ITD sensitivity under CI stimulation in a new animal model of neonatally deafened rats and compared these to hearing experienced rats. Stimuli were designed to sample

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the physiologically relevant ITD range for this species at a resolution fine enough to resolve the animals' known behavioral ITD thresholds and the analysis methods included onset responses, thought to be the most salient. In addition, we have concluded our analysis in a manner that facilitates comparison to behavioral thresholds.

2. Methods and Materials

All procedures involving experimental animals reported here were performed under license issued by the Department of Health of Hong Kong (#16-52 DH/HA&P/8/2/5) and approved by the City University of Hong Kong Animal Research Ethics Sub-Committee.

2.1. Subjects & Deafening

A total of twelve wild type female Wistar rats were used in this study to investigate ITD sensitivity in the inferior colliculus under bilateral CI stimulation. Four animals were raised to early adulthood with normal hearing experience. The remaining eight rats were deafened neonatally using kanamycin injection protocols to induce cochlear hair cell loss prior to the onset of hearing, as described previously (Rosskothen-Kuhl and Illing 2010; Rosskothen-Kuhl and Illing 2012; Rauch et al. 2016; Rosskothen-Kuhl et al. 2018). Each of these eight animals had daily kanamycin sulfate (Sigma, 400 mg/kg body weight) intraperitoneally injections from postnatal day 9 to 20, inclusively. This method results in widespread death of inner and outer hair cells (Matsuda et al. 1999). Osako et al. (1979) have shown that rat pups treated with this method never achieve hearing thresholds below 70 dB SPL during very early infancy (~p14-16), after which they are severely to profoundly hearing impaired with thresholds above 95 dB SPL. This early deafening results in widespread modifications in the development of the central auditory pathways histologically. These modifications include: changes in molecular, cellular, and morphological properties, including a massive increase and broadening of neuronal activation patterns which indicates a degraded tonotopic organization (Rosskothen-Kuhl and Illing 2012; Rauch et al. 2016; Jakob et al. 2019). In the inferior colliculus, this neuronal response was accompanied by a massive hypertrophy of

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astrocytes and microglia and an augmentation of the GABAergic neuronal network (Rosskothen-Kuhl and Illing 2012; Rauch et al. 2016; Rosskothen-Kuhl et al. 2018). The Preyer's reflex, a motor reflex to a loud hand-clap, was checked daily with each kanamycin injection and was only present between ~p14-16 (Jero et al. 2001). In addition, hearing loss was confirmed by measuring auditory brainstem response (ABR) thresholds to broadband click stimuli up to 90 dB SPL or higher prior to implantation in early adulthood.

2.2. Cochlear Implantation & Craniotomy

All animals in this study were implanted with bilateral CIs in early adulthood (~p64-77) and recorded the same day. All surgeries and recordings were conducted under anesthesia, which was induced by intraperitoneal (i.p.) injection of ketamine (Alfasan International B.V, 80mg/kg) and xylazine (Alfasan International B.V, 12 mg/kg), and maintained with an infusion pump delivering 17.8 mg/kg/h ketamine and 2.7 mg/kg/h xylazine in 0.9 % saline i.p. at a rate of 3.1 ml/h. Body temperature was kept constant at 38°C using a feedback-controlled heating pad (RWD Life Sciences, Shenzhen, China). A midline scalp incision was made to expose the skull, and craniotomies were performed just anterior to lambda and just lateral to the midline suture to expose the occipital cortex that covers the dorsal surface of the inferior colliculus. All neonatally deafened animals had bilateral craniotomies over both inferior colliculi while hearing experienced animals had only one craniotomy over the right inferior colliculus. All animals then received binaural cochlear implants. Detailed descriptions of our cochlear implantation methods can be found in (Rosskothen-Kuhl and Illing 2010; Rosskothen-Kuhl and Illing 2014; Rosskothen-Kuhl and Illing 2015; Rauch et al. 2016; Rosskothen-Kuhl et al. 2021). In short, four rings of an eight channel intracochlear electrode array (ST08.45, Peira, Beerse, Belgium) were fully inserted through a cochleostomy window into the middle turn of each cochlea. The arrays were directed towards the apical cochlea so that the tip electrode, used for intracochlear stimulation, sits approximately in the

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4-8 kHz region. This CI insertion method is highly reproducible, and places the electrodes in a range that would normally also be covered by clinical electrode arrays inserted in human CI patients and does not specifically target apical regions of the cochlea. Although ITD sensitivity is traditionally thought to be a spatial cue for low-frequency signals, recent psychoacoustic studies in human CI users did not in general find lower ITD thresholds when more apical regions of the cochlea were stimulated (Kan et al. 2015). In our animal model we target a part of the cochlea which would routinely be covered by clinical implants for better translation to human CI users.

Our cohort of normal hearing animals was not chemically deafened prior to implantation and recording, although their tympanic membrane and middle ear ossicle chain were removed in the process of exposing the inner ear for cochleostomy. This would lead to substantial conductive hearing loss, and no acoustic stimuli were presented during the experiments. Nevertheless, this leaves open the possibility that these animals therefore received some “electrophonic” stimulation through surviving hair-cells, so the nature of the CI stimulation of their auditory nerves will likely have differed in subtle ways from that of the neonatally deafened cohort. However, our practice here is in keeping with current clinical practice in human patients, where one tries to encourage post-implantation hair cell survival where possible (von Ilberg et al. 1999; Gstoettner et al. 2006; Turner et al. 2010). Animal studies on electro-acoustic hearing suggest that, if anything, electrophonic responses would result in mild suppression and minimal distortion of the auditory nerve fiber responses (Tillein et al. 2015). Moreover, no hair cell excitation occurs when presenting electrical stimulation in the context of electro-acoustic masking (Imsiecke et al. 2020). In any event, it is unlikely that electrophonic hearing could have any major effects on ITD encoding in our study. Any physiological delays or changes in the temporal pattern of nerve fiber discharges induced by electrophonic stimulation would be expected to be symmetric in both ears and therefore independent of interaural delays. Even if there was some left-right asymmetry in the evoked responses, such an asymmetry could only add a constant offset to the ITDs, but would not change

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the way changes in stimulus ITD are reflected in changes in auditory nerve firing patterns. It is therefore very hard to see how the amount of ITD tuning observed, that is, the extent to which changes in stimulus ITD are reflected in changes in IC neuron response amplitudes, could be affected by or be due to the presence of electrophonic stimulation.

2.3. ABR and eABR recording

To verify that hearing experienced animals did in fact have normal hearing thresholds and neonatally deafened animals had threshold above 90 dB SPL, ABRs were measured prior to implantation in all animals. The recording procedure is described in Rosskothen-Kuhl et al. (2018): under ketamine (80mg/kg) and xylazine (12 mg/kg) anesthesia, each ear was stimulated separately through hollow ear bars with 0.5 ms broad-band clicks with peak amplitudes up to 130 dB SPL, delivered at a rate of 43 Hz. ABRs were recorded by averaging scalp potentials measured with subcutaneous needle electrodes between mastoids and the vertex of the rat's head over 400 click presentations. Examples for each cohort are shown in Figure 1A and B. Following CI surgery, electrically evoked ABRs (eABRs) were measured for each ear individually to verify that both CIs were symmetrically implanted and operated at acceptably low electrical stimulation thresholds, usually around 100 µA. eABRs were recorded before and after inferior colliculus recordings as described in (Rosskothen-Kuhl et al. 2021). eABR thresholds are shown in Table 1 and an example recording in Figure 1C.

Animals	Left eABR threshold (dB re 100 µA)	Right eABR threshold (dB re 100 µA)
Neonatally Deafened 1	5	2.5
Neonatally Deafened 2	2	0
Neonatally Deafened 3	2.5	2.5

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Neonatally Deafened 4	5	0
Neonatally Deafened 5	7.5	7.5
Neonatally Deafened 6	4	8
Neonatally Deafened 7	7.5	7.5
Neonatally Deafened 8	5	2.5
Hearing Experienced 1	0	0
Hearing Experienced 2	2.5	2.5
Hearing Experienced 3	2.5	2.5
Hearing Experienced 4	5	0

Table 1: Overview of the left and the right electrically evoked auditory brainstem response (eABR) thresholds of all CI animals.

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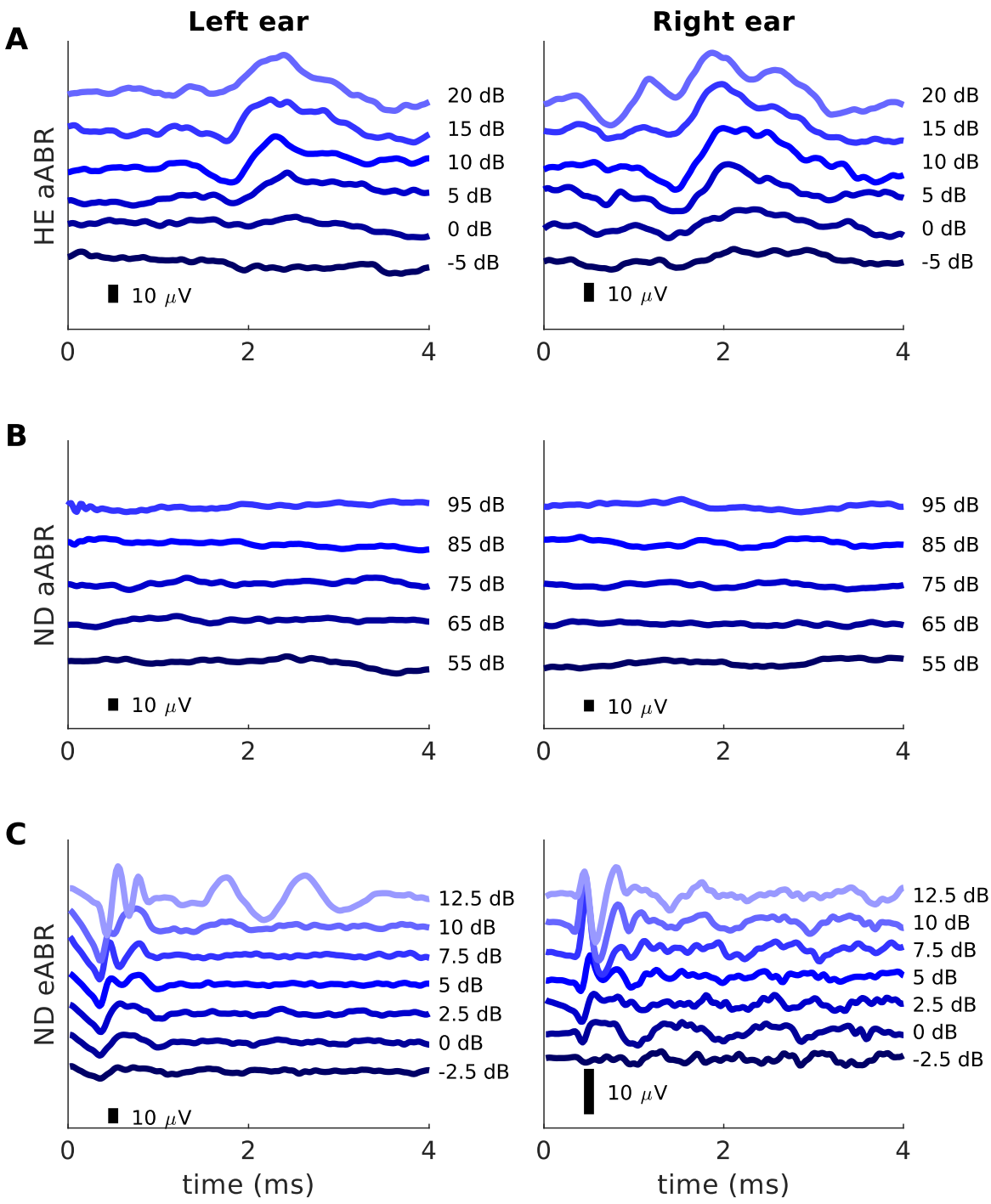

Figure 1: Examples of auditory brainstem responses (ABRs) of hearing experienced (HE) and neonatally deafened (ND) animals. Responses are shown in left and right columns for the left and right ears, respectively. Sound intensities are shown to the right of each plot. A and B show

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acoustically evoked ABRs (aABRs) with broadband click presentation at the respective SPL levels. A: aABRs for a hearing experienced animal prior to implantation. B: aABRs for a neonatally deafened animal prior to implantation. C: electrically evoked ABRs (eABRs) for a neonatally deafened animal post implantation with stimulation levels in relation to 100 μA (see method 2.4 for details).

2.4. Electric intracochlear stimulation and multi-unit recordings

All stimuli were presented using a Tucker Davis Technology (TDT, Alachua, Florida, US) IZ2MH programmable constant current stimulator at a sample rate of 48,828.125 Hz thus allowing for a minimum sample duration of 20.48 μs. The most apical ring of each CI electrode array served as the stimulating electrode and the second ring as the ground electrode. The remaining rings on the array were not used in these experiments. All electrical intracochlear stimuli consisted of single, binaural, biphasic, anode leading current pulses similar to those used in clinical devices (duty cycle: 61.44 μs positive, 40.96 μs at zero, 61.44 μs negative). Stimulus amplitude in each ear was held constant at a value of 5-10 dB above eABR thresholds, corresponding to typical amplitudes in the order of ~200-600 μA. We report CI stimulus dB values as $20 \cdot \log_{10}(A/A_{ref})$ where A and A_{ref} are peak amplitudes of the biphasic pulses, and A_{ref} is either the threshold amplitude, or a reference amplitude of 100 μA or as indicated.

To deliver ITDs on the binaural stimuli, the pulses were delayed in one ear relative to the other by an integer number of samples, enabling us to vary ITDs in steps of 20.48 μs. Stimuli consisted of a single pulse in each ear. In four neonatally deafened rats, we recorded responses with ITDs in single sample steps, covering the values ± {0, 20.48, 40.96, 61.44, 81.92, 102.40, 122.88, 143.36, 163.48 } μs. For simplicity, and given that ITD changes of less than 4 μs are well below any physiological or psychoacoustic threshold ever reported, we report ITDs below rounded to the nearest 10 μs, i.e. as ± 0, 20, 40, 60, 80, 100, 120, 140, or 160 μs. Each ITD value was presented 30 times at each recording site, in a pseudo randomly interleaved order. Inter-trial intervals were approximately 500 ms, with some variability given that the software controlling the stimulus delivery

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was not a real time system. In this manner we collected ITD responses in steps fine enough to resolve the animal's known behavioral threshold of ~50 µs (see Supplementary Fig. 1; Li et al. 2019; Rosskothen-Kuhl et al. 2021). The chosen range of ITDs was slightly wider than the rats' physiological ITD range (~± 120 µs, Koka et al. (2008)), and by sampling with fine-grained ~20 µs steps we placed 13 ITD values within the rat's physiological ITD range. The remaining four neonatally deafened animals were subsequently tested with a larger ITD range (±300 µs) and with wider steps of ~75 µs, similarly to what had been done in previous studies by other authors who mostly used steps of 100 µs or greater and typically only included a minority of sample points (between 1 and 7) within the physiological ITD range of the respective model species (~±400 µs for cats and ~±300 µs for rabbits) (Day et al. 2012). For details on the calibration which confirmed that our CI setup delivered the desired ITDs and no usable intensity cues, see Rosskothen-Kuhl et al. (2021). In this paper we report ITD values as negative if the ITD is leading in the ear contralateral to the inferior colliculus from which recordings were taken, and as positive where the ear ipsilateral to the recording site is leading. In doing so we follow a long established and common convention in the sound localization literature to use negative values to denote contralateral space (Yin and Chan 1990; Middlebrooks et al. 1998; Mrsic-Flogel et al. 2003; Campbell et al. 2006; Tollin and Yin 2009), but we acknowledge that the opposite convention is also common.

Multi-units were recorded using a single-shaft, 32-channel silicon electrode array (ATLAS Neuroengineering, E32-50-S1-L6/L10), which was inserted into the inferior colliculus through a craniotomy exposing the overlying occipital cortex while the anesthetized animal was fixed in a stereotaxic frame within a sound attenuating chamber. Both, the left and right, inferior colliculi were targeted using stereotaxic coordinates and anatomical landmarks around the sagittal sinus. Penetration locations were chosen so as to sample the stereotactic area of interest extensively and fairly evenly while avoiding blood vessels or other potential obstacles, and observing a minimum distance of 0.5 mm from previous penetration sites. For each penetration, the tip of our electrode was initially advanced to a depth of 4.5 mm from the brain surface, and then slowly advanced

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further while monitoring the electrodes for responses to isolated “search stimulus” CI pulses delivered at ~ 1 Hz. Extracellular recordings were made using TDT equipment at a sampling rate of 25 kHz. Brainware software with custom stimulus scripts was used to deliver the stimuli and acquire the electrophysiological data. All experiments were terminal.

2.5. Analysis

All data processing and analysis was performed using custom code written in Matlab R2018b. Analog multi-unit activity (AMUA) was computed from the recorded extracellular voltage traces as described in (Kayser et al. 2007; Schnupp et al. 2015). This method quantifies neural activity based on the amplitude envelope of the electrode signal in the frequency band occupied by action potentials. To compute the AMUA, electrode signals were bandpass filtered using a 4th order butterworth filter (0.3-6 kHz), the absolute value was taken, followed by further lowpass filtering (6 kHz). The resulting AMUA trace served as a measure of local multi-unit firing rates that is usually less noisy than multi-unit activity measures based on threshold crossings (see Fig. 1 in Schnupp et al. (2015)). This is due to the fact that thresholding itself can introduce quantization noise. For this reason, we used threshold crossings (three standard deviations below the mean of the 0.3-6 kHz band-passed signal) only for the raster plot visualizations and the comparison of spike count and AMUA amplitude based tuning curves shown in Figure 2. Statistical analyses were all performed on AMUA amplitudes. Responses to stimuli were then quantified by computing the mean amplitude of the AMUA signal during a response period set to be 2.8-40 ms post stimulus onset, and subtracting the mean baseline amplitude computed over a period of 300 to 500 ms after stimulus onset. For brevity we shall refer to this baseline corrected mean AMUA response amplitude as “AMUA response” below. No evoked neuronal responses were expected or observed at latencies shorter than about 3 ms in the inferior colliculus. Note that our analysis quantifies “onset” ITD responses, which are known to be the most salient in behavior (Brown and Stecker 2010) and in

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physiological measures (Greenberg et al. 2017). This differs from previous studies which focused on steady-state and sustained ITD responses (Hancock et al. 2010; Hancock et al. 2012; Hancock et al. 2013), and in which onset ITDs were intentionally excluded. Our electric intracochlear stimuli were single binaural pulses of less than one ms duration (see above) and their electrical artifacts had died down completely before the start of our analysis time window, so we were able to remove electrical stimulus artifacts simply by "blanking" the recordings over the period of 0-2.8 ms post stimulus onset.

To quantify the ITD sensitivity of neurons in the inferior colliculus, we computed the mutual information (Mrsic-Flogel et al. 2003; Nelken et al. 2005; Gordon et al. 2008) between AMUA responses and stimulus ITD. Mutual information quantifies the statistical interdependence between neural response and stimulus parameter in bits per response. AMUA responses were discretized into seven levels, and the adaptive-direct method described by Nelken et al. (2005) was used to estimate mutual information values from neural response distributions, as well as to determine whether mutual information values (after bias correction) were significantly greater than zero. The statistical significance was assessed, and values were bias corrected, by the commonly used method of performing a permutation test, in which responses were randomly reassigned to different ITD values, allowing us to quantify the amount of mutual information we would expect to see by chance. This random shuffling of responses was repeated 100 times, and the mean mutual information value from the shuffled responses then served as estimate of the bias of the raw mutual information value, and the 99^{th} centile served as critical value for the permutation test with $\alpha=0.01$. Only multi-units with mutual information values significantly above zero were deemed ITD sensitive and included in further analysis. In addition, a linear mixed effects model was used to determine if the difference between groups was statistically significant. We have included penetration identity as a random effect to account for the non-independent sampling of neighboring electrodes.

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To determine the tuning of these ITD sensitive multi-units we used a principal component analysis in which four statistically independent clusters were identified in the pooled normal hearing experienced and neonatally deafened cohorts according to Euclidean distances (Fig. 5 A-C). Prior to principal component analysis, the responses for each tuning curve were "centered" by subtracting their mean and normalized by their standard deviation effectively calculating z-scores. This normalization step makes the analysis insensitive to possible confounding effects of differences in overall response amplitudes, rather than tuning curve shape. Principal components were then subjected to hierarchical clustering and distributions of clusters per cohort and animals were then determined.

To evaluate sample bias effects we bootstrapped 1000 "simulated ND" and 1000 "simulated HE" cohorts each consisting of 4 animals, by drawing sets of 4 values from the tuning distribution proportions in the set {0, 38, 79.4, 68.9, 85.8, 97.6, 97.7, 100} for each simulated cohort, with replacement. These values were taken from the distribution of contralateral tuning determined from the analysis above. We then compared the average contralateral tuning percentage seen in each pair of simulated cohorts in order to evaluate whether the tuning distribution could be due to a sample bias.

Finally, in an attempt to quantify how observed differences in ITD tuning curve shapes between the different cohorts might influence the ability to perform a left-right two-alternative forced choice ITD discrimination task, we calculated neural d-prime (*d'*) values. Our approach is inspired by (Shackleton et al. 2003), who computed ROC values from neural response data in order to make these more directly comparable to psychophysical performance measures. The approach is equivalent since, in psychophysical signal detection theory, ROC and *d'* are linked via the relationship $d' = \sqrt{2}\,Z(ROC)$ where Z() is the inverse cumulative normal distribution. In essence, *d'* quantifies how far apart the means of two distributions are in multiples of their standard deviations. It thereby quantifies the discriminability of responses drawn from the distributions as an inverse relation to the overlap between the distributions. Here, we considered the contralateral and

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ipsilateral AMUA response distributions for paired ITD values in order to measure the discriminability of neural responses across the 30 trials for each ITD. Following a convention established by Hancock et al. (2010), we treat cases as a “hit” where the response to the contralateral stimulus was strongest and as a “false alarm” where the ipsilateral response was strongest, irrespective of the tuning curve shape. The mean values and variances for the contra- and ipsilateral segments of the AMUA responses for symmetric ITD values in each trial are taken in order to calculate the *d′* value so that:

$$d' = \frac{mean(ipsi) - mean(contra)}{\sqrt{(0.5(var(ipsi) + var(contra)))}} \qquad (1)$$

where ‘contra’ and ‘ipsi’ are the set of AMUA responses across the 30 trials recorded for a given ITD for either the contralateral or the ipsilateral ear leading, respectively. Consequently, multi-units with predominantly ipsilateral tuning will score *d′* values above 0. Note that absolute *d′* values of ≥1, are equivalent to performances in a two alternative forced choice task that exceeds 75% correct, and can serve as a useful “performance threshold” (see Fig 6). Note that the use of Eqn 1 for computing *d’* is highly computationally efficient, but may not be suitable for signals with a highly non-normal distribution, in which case the ROC method introduced by Shackleton et al. (2003)may be preferable. We verified that, for our data, Eqn 1 gives very similar results to those obtained when computing ROC values using the Shackleton et al. (2003) method and then converting them to *d′*.

2.6. Code accessibility

All data and custom code will be made available upon request.

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3. Results

Using the methods just described, we recorded responses from a total of 12 animals, four hearing experienced finely sampled, four neonatally deafened finely sampled, and four neonatally deafened coarsely sampled animals. The breakdown of how many penetrations were sampled from each animal is given in Table 2. In total, we recorded from 106 penetrations with a 32 multi-channel electrode, and our total dataset therefore comprises 3392 recording sites. One-way ANOVA on response amplitudes during the response window (2.8-40 ms post stimulus onset) against baseline (alpha=0.01) confirmed that all 3392 recording sites exhibited evoked responses to the CI stimulation, and AMUA response amplitudes were therefore computed as described for all recording sites.

Animal ID	# Penetrations in right IC	# Penetrations in left IC	Sampling of ITD tuning curves (fine or coarse)
Hearing Experienced 1	13	0	fine
Hearing Experienced 2	8	0	fine
Hearing Experienced 3	9	0	fine
Hearing Experienced 4	11	0	fine
Neonatally Deafened 1	6	8	fine
Neonatally Deafened 2	4	5	fine
Neonatally Deafened 3	3	6	fine
Neonatally Deafened 4	3	3	fine
Neonatally Deafened 5	3	0	coarse
Neonatally Deafened 6	10	0	coarse
Neonatally Deafened 7	9	0	coarse
Neonatally Deafened 8	5	0	coarse

Table 2: Overview of number of penetrations in the left or right inferior colliculus (IC) of both cohorts. Also shown is whether ”fine grained” ITD tuning curves from -160 to +160 µs in 20 µs steps or “coarse grained” tuning curves with ITDs ranging from -300 to +300 µs in 75 µs steps were sampled.

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3.1. ITD sensitivity exists in both neonatally deafened and hearing experienced animals, but with differing patterns

Figure 2 shows representative examples of individual multi-unit raster plots and corresponding tuning curves of bilaterally CI stimulated, neonatally deafened animals (left) and hearing experienced animals (right). In each raster plot, alternating horizontal bands of shading separate each of the 17 ITDs tested. Each band of ITD consists of 30 repeated presentations stacked vertically. The response window shown excludes the first 2.8 ms to blank the electric artifact. Most multi-units showed initial onset responses at around 5 ms after stimulus onset, as well as secondary responses peaking at around 10-20 ms post stimulus onset. The tuning curves shown have been normalized relative to their maxima for ease of comparison. As previously described for other animal models (Hancock et al. 2010; Tillein et al. 2010; Hancock et al. 2013; Tillein et al. 2016; Vollmer 2018; Chung et al. 2019), we observed various neuronal ITD tuning shapes such as “peak”, “trough”, “multi-peak”, all within the physiological range of rats.

The illustrative examples shown in Figure 2 were selected to show a range of responses varying in the “strength” of ITD tuning as quantified by the mutual information between single trial AMUA response amplitudes and stimulus ITD, as well as to illustrate some of the variation in tuning curve shapes observed. The mutual information values increase from top to bottom, and multi-units were selected to give examples of comparable mutual information values between the neonatally deafened and hearing experienced datasets. Also shown for comparison are tuning strengths computed as signal-to-total-variance-ratios (STVRs, also sometimes referred to as signal-to-noise-ratios SNRs), a metric favored by some other authors (Hancock et al. 2010; Hancock et al. 2012). We show two versions of the computed tuning curves for each multi-unit in Figure 2: one computed with traditional spike counting after spike detection by thresholding (light colored lines) and one computed using our preferred AMUA method described above (darker lines). It is readily apparent that the general shape of the tuning curves is very similar for both metrics. In some of the

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examples, the AMUA method gives tuning curves that may seem a little more shallow, with less pronounced dips for less effective ITDs, but it makes up for that with much smaller error bars, indicating substantially lower trial-to-trial variability in the responses.

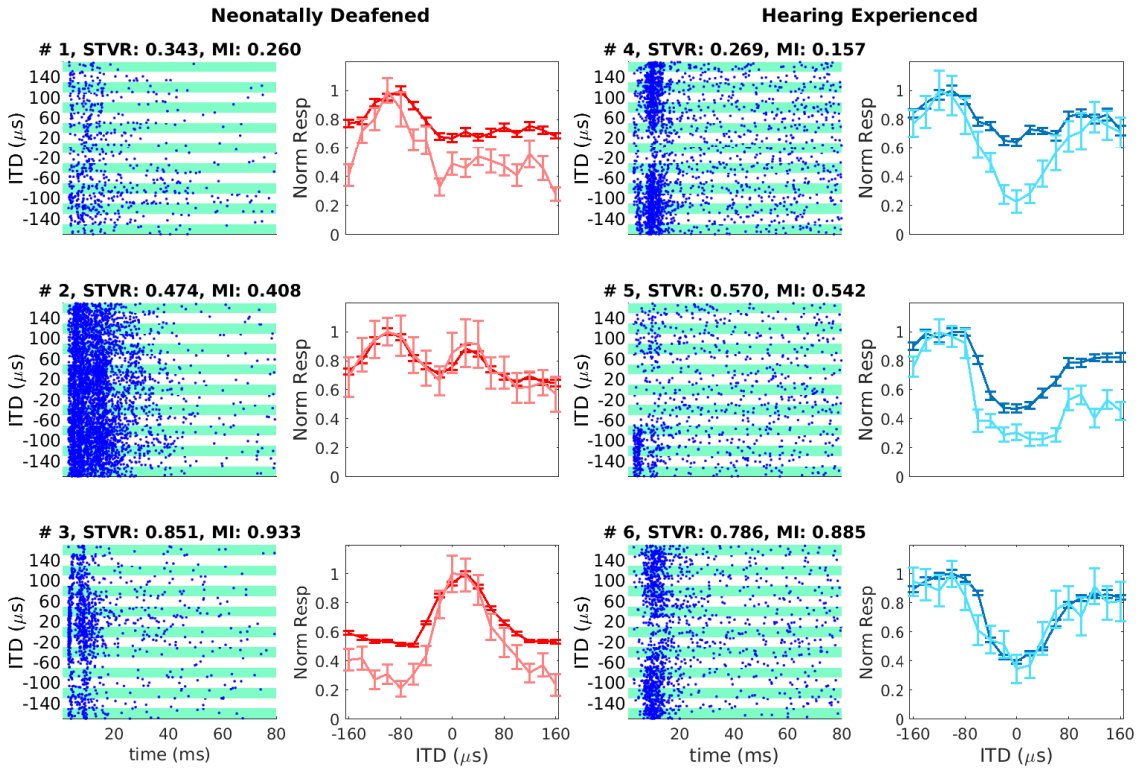

Figure 2: Spike raster plots and tuning curves for representative example multi-units for neonatally deafened (left columns) and hearing experienced (right columns) animals. Each blue dot represents a spike, successive rows of dots show responses to repeated presentations of stimuli. Responses for different ITD values are indicated by the alternating white and light green backgrounds. Multi-units are arranged from top to bottom in order of increasing ITD sensitivity, as quantified by higher mutual information (MI). The “signal-to-total-variance-ratio” (STVR) and corresponding MI value, in bits/response, are shown above each panel for each multi-unit. The tuning curves for these same units (red for neonatally deafened rats, blue for hearing experienced rats) are plotted with error bars showing the standard error of the mean response amplitude calculated across repeated presentations for each ITD value. Tuning curves in red and dark blue are computed from AMUA response amplitudes, those in pink or light blue are from multi-unit spike counts determined by simple thresholding. For these tuning curve plots, responses were baseline corrected and normalized relative to the maximal response. Negative ITD values indicate that pulses are earlier in the ear contralateral to the recording site.

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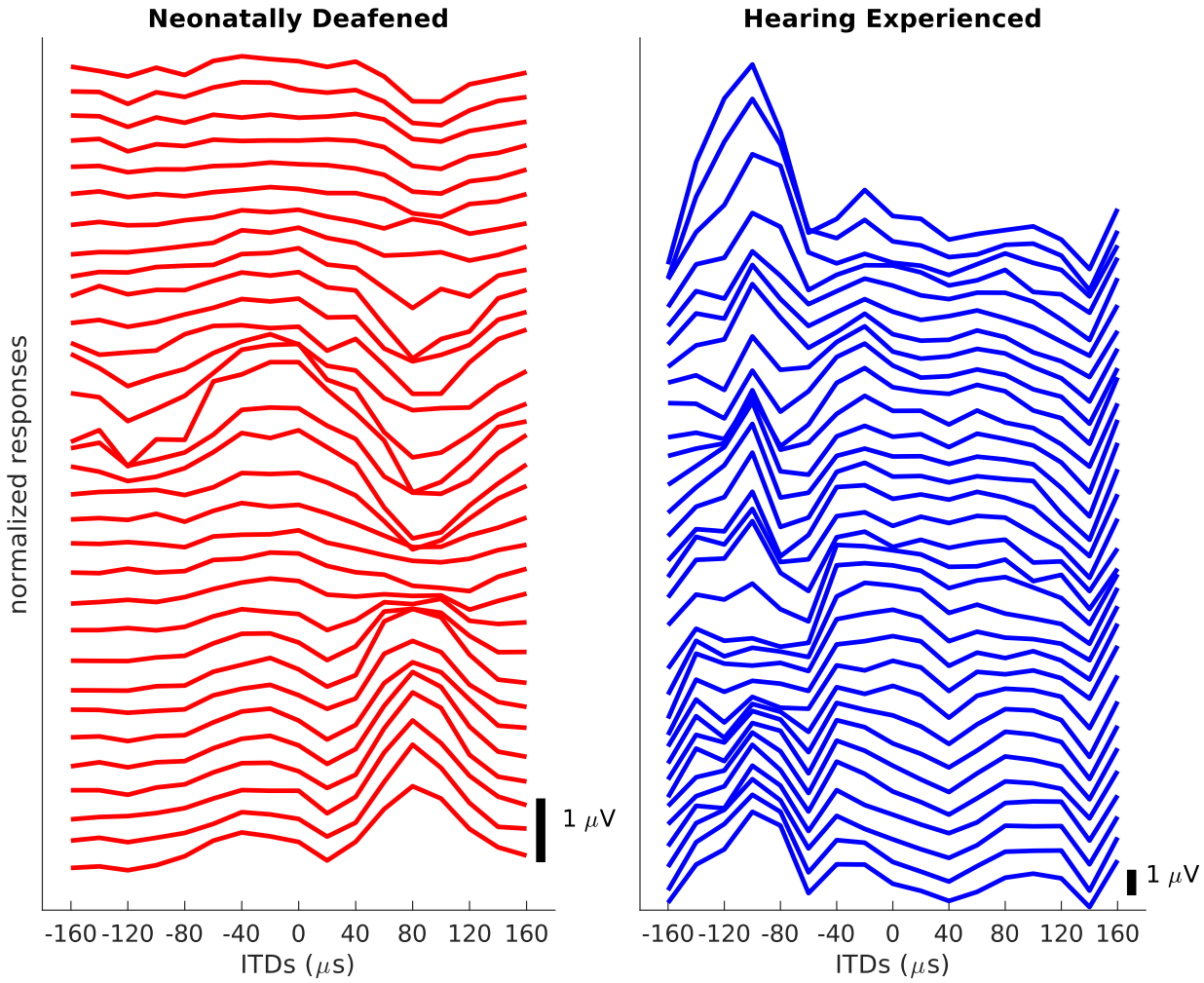

Figure 3: AMUA ITD tuning curves recorded along the 32 recording sites of a single vertical multi-electrode penetration into the inferior colliculus of a neonatally deafened (left) and a hearing experienced (right) animal. Scale bars for 1 μV are shown to the right of each subplot.

Tuning properties for neurons in inferior colliculus (as well as many other sensory structures) tended to "cluster" in the sense that anatomically neighboring neurons are expected to have more similar tuning curves than two neurons chosen at random (Schnupp et al. 2015; Li et al. 2019). Consequently, neighboring multi-units that were simultaneously recorded along a single multi-channel electrode may not safely be considered "independent observations" for the purposes of statistical testing. To illustrate that this is a relevant factor in our dataset, and to give some

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examples of how similar or dissimilar tuning-curves along a single multi-channel electrode penetration may be, we give in Figure 3 examples of two 32-channel electrode penetrations, one from each cohort, showing tuning curves recorded at 0.05 mm intervals along the dorso-ventral axis. As we will see below, these examples are somewhat “typical”, in that the tuning curves seen in hearing experienced recordings (Fig. 3, right plot) were predominantly contralaterally peaked, while those in the neonatally deafened animal were more diverse and were more likely to exhibit high levels of activity for central or ipsilateral ITDs.

3.2. Multi-units in neonatally deafened rats were on average no less ITD sensitive than those in hearing experienced rats

To compare the strength of ITD tuning in hearing experienced and neonatally deafened animals, we examined the distributions of mutual information values between AMUA response and ITD for both cohorts tested with finely sampled ITDs. These distributions are shown in Figure 4A and B, respectively. Different shading is used to indicate whether the mutual information value for a given multi-unit was significantly greater than zero (dark green bars), as determined by the permutation test described in methods. In total, 82.5% (1081/1311) of the inferior colliculus multi-units from hearing experienced animals and 84.8% (966/1139) from neonatally deafened animals showed statistically significant (α=0.01) amounts of mutual information between ITD value and neural response. The proportions of ITD sensitive units for each neonatally deafened animal were ND1: 268/419 = 63.93%, ND2: 270/270 = 100%, ND3: 256/270= 94.81, and ND4: 174/180= 96.67%. Likewise for each hearing experienced animal the proportions of ITD sensitive units were HE1: 121/256 = 47.27%, HE2: 278/288 = 96.53%, HE3: 336/416 = 80.77% and HE4: 348.99/350 = 99.71%. We observed a higher number of units with relatively large MI values in the neonatally deafened rats (Fig. 4A) than in the hearing experienced animals (Fig. 4B), but the two distributions

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substantially overlapped, and the overall number of units showing significant ITD sensitivity was comparable between the two groups.

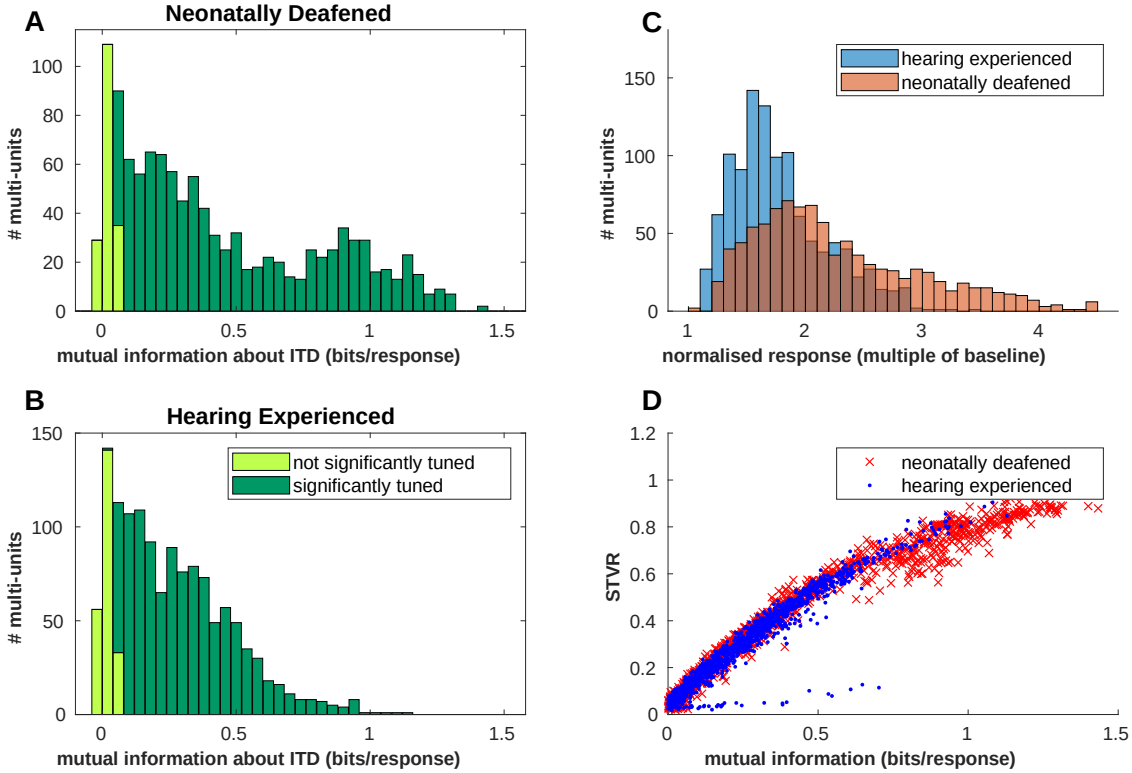

Figure 4: Mutual information between ITD and responses of inferior colliculus multi-units. A, B: Stacked bar charts showing the distribution of mutual information values between ITD and neural response in bits/response for multi-units recorded in neonatally deafened (A), and hearing experienced (B) rats. Multi-units with mutual information values significantly above zero are shown in dark green, those failing to reach significance in light green. C: Histograms of peak response amplitudes, quantified as multiples of baseline activity, for multi-units with significant ITD tuning based on mutual information values for neonatally deafened rats (red) and hearing experienced rats (blue). D: Mutual information values correlated highly with signal-to-total-variance-ratio (STVR) values for our multi-unit data.

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In order to determine whether the apparent differences in the distributions of the mutual information values for the two cohorts (Fig. 4A, B) were statistically significant, we log transformed the mutual

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information values for all units to obtain a more normally distributed outcome variable, and used a linear mixed-effects model factor to test whether hearing experience had a significant effect on the average log(mutual information). The mixed-effects model took into account that simultaneously recorded multi-units from a single 32 multichannel electrode penetration cannot be considered independent observations, by treating the 79 multi-channel electrode penetrations performed with fine sampling in this study as a random effect. The model formula was therefore:

$$log(mutual\ information) \sim 1 + hearing\ experienced + (1 \mid penId) \quad (2)$$

where *hearing experienced* is an index variable giving hearing experience status (0 for neonatally deafened rats or 1 for hearing experienced rats), and *penId* is an index variable for penetration number, which groups all multi-units from the same penetration and removes systematic differences from one penetration to another. The model confirmed that *hearing experience* had a significant effect on mutual information values with $p = 0.017$. This can also be appreciated with the different shapes of the mutual information histograms shown in Figure 4A and B. However, note that the mixed-effects model does not take into account the possibility of "nested" dependencies of penetrations within individual animals and it may therefore overestimate significance levels. At the time of writing, we were unable to find a statistical library that offers nested mixed-effect linear model fits to continuous valued data. Ultimately, we are not too concerned about whether the neonatally deafened cohort had significantly higher mutual information than the hearing experienced one. What we can say with certainty though, is that the mutual information in the deafened animals was not lower, which in itself is an interesting and perhaps surprising result given numerous other studies mentioned in the introduction which have documented difficulties with ITD sensitivity in deafened patients or animals.

We also examined whether there were any trends for mutual information values to increase or decrease systematically over the course of each recording experiment, as that might have indicated an instability or gradual deterioration in the physiological condition of the animals. No

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systematic relationship was found between mutual information values and time of recording relative to the start of the experiment.

Figure 4C compares response amplitudes in the two cohorts, showing histograms of maximum response values expressed as multiples of the baseline amplitudes. For this Figure, expressing peak response amplitudes as multiples of the baseline activity observed at each recording site was done in order to make this comparison less sensitive to changes in electrode impedances that are to be expected from site to site and from animal to animal, and which would affect the recorded voltages, but should not change the factor by which they increase following stimulation. It is clear that responses to CI stimulation are on average stronger in the inferior colliculus of the neonatally deafened cohort as compared to the hearing experienced group. In Figure 4C we see that almost a quarter of multi-units of the neonatally deafened cohort exhibited peak responses more than three times that of the baseline activity, while in the CI-stimulated hearing experienced cohort peak responses greater than three times that of the baseline were very rare. The median peak response for multi-units in the inferior colliculus of neonatally deafened rats was 2.2 times greater than baseline responses, compared to 1.7 times seen in multi-units of hearing experienced animals. These differences were statistically significant ($p < 10e-6$), as determined by a linear mixed-effects model equivalent to that used above to test for differences in mutual information between cohorts. Note that the amplitudes of stimulus pulses used, as well as eABR thresholds, were comparable between cohorts, suggesting these differences cannot be explained by simple, systematic differences in stimulation intensities.

From these data we conclude that ITD tuning in our neonatally deafened cohort was comparable to that in the hearing experienced cohort. This is a surprising result in light of several studies which have documented reduced ITD sensitivity in deafened animals. One set of studies which described reduced ITD sensitivity in deafened cats (Hancock et al. 2010; Hancock et al. 2012) uses a metric known as "signal to noise ratio" or later, perhaps more accurately, as "signal-to-total-variance-

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ratio". We have compared this signal-to-total-variance-ratio to our measure of ITD sensitivity, mutual information, in Figure 4D. From this we can clearly see that the two measures correlated closely for both cohorts and thus our results are not a consequence of our choice of ITD sensitivity measure.

3.3. Distributions of ITD tuning curve shapes differed between hearing experienced and neonatally deafened animals

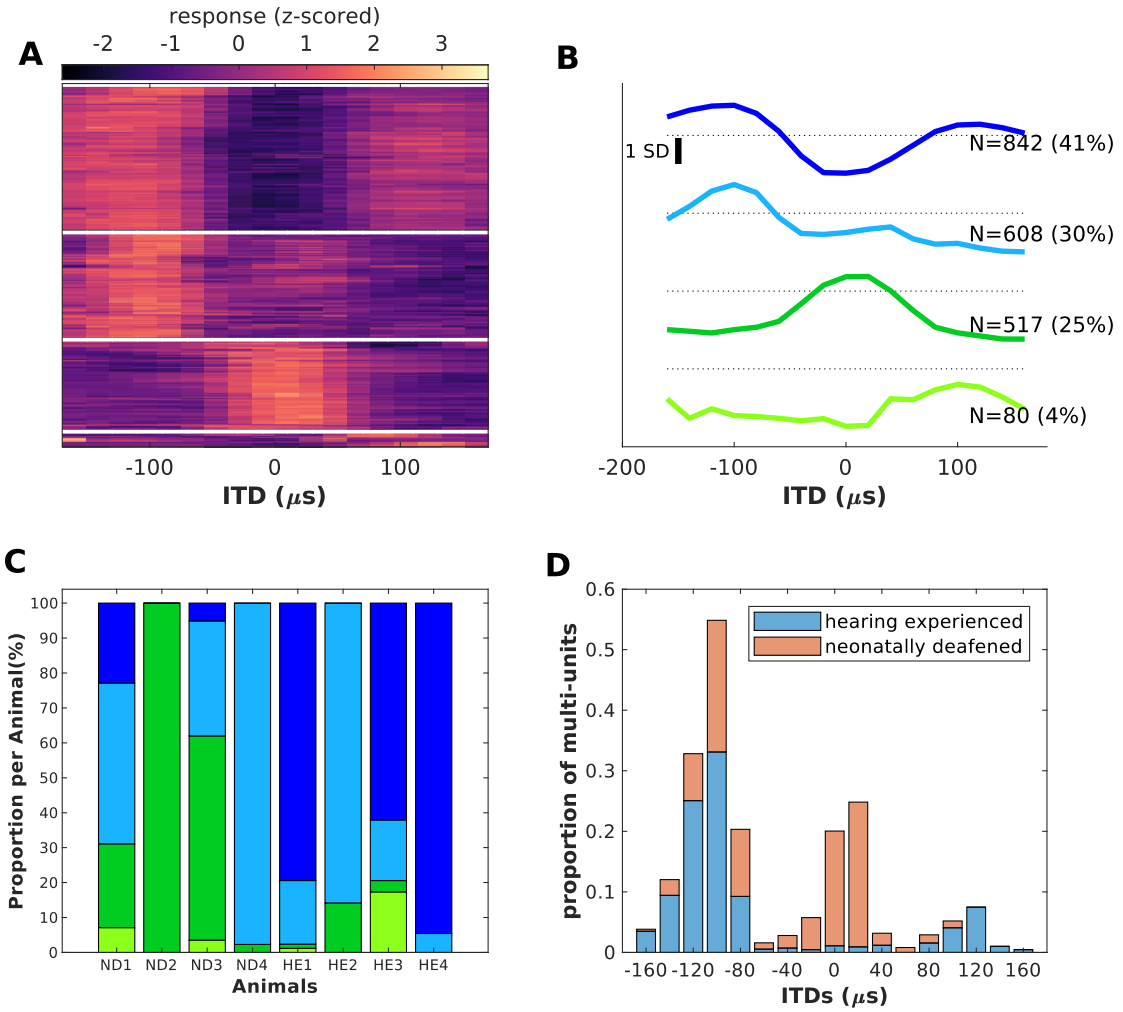

Figure 5: Distribution of ITD tuning curve shapes differ systematically between normal hearing experienced (HE) and neonatally deafened (ND) rats. A: Tuning curves of all multi-units with significant ITD tuning, shown as a heat map, with tuning curves sorted into four clusters determined by a hierarchical clustering algorithm (see methods). B:

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Mean tuning curve for each of the four clusters shown in A. Responses in the tuning curves were normalized to unit standard deviation. Scale bar shows 1 standard deviation (SD). The total number of multi-units, and its percentage, are shown next to each curve. C: Stacked bars showing the distribution of tuning curve types (clusters) for the four neonatally deafened animals (ND1-ND4) and the four hearing experienced (HE1-HE4) animals with finely sampled ITDs. Colors match those of the mean tuning curves for the clusters shown in B. D: Stacked histograms of peak ITD values for all multi-units from neonatally deafened (salmon-pink) and hearing experienced (blue) animals.

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The data in Figure 4 show that the strength of tuning to ITD, as quantified by the mutual information between neural responses and stimulus ITD, was on average, no worse in our neonatally deafened rats compared to their hearing experienced peers. However, similar proportions of ITD sensitive units do not imply that ITD tuning curve shapes are also similar 465 between the two cohorts. The examples from Figures 2 and 3 show that ITD tuning curve shapes in the inferior colliculus in response to electrical stimulation can be quite diverse, a fact that has also been reported previously (Hancock et al. 2010; Tillein et al. 2010; Chung et al. 2019; Rosskothen-Kuhl et al. 2021). In order to impose an order on the diverse tuning curve shapes in a data-driven manner, we subjected them to a cluster analysis. The tuning curves for all significantly 470 tuned multi-units were pooled across both cohorts (n=2047), with their mean subtracted and normalized by their standard deviation resulting in z-scores, and subjected to principal component analysis followed by hierarchical clustering. The first five principal components were found to account for 90.36% of the variance in tuning curve shapes. We therefore represented each tuning curve by the first five principal components, and subjected these vectors to hierarchical clustering 475 using the Matlab function “cluster()” with Euclidean distance metrics and complete linkage. This categorized the tuning curves into four distinct clusters shown in Figure 5A which accounts for 66.7% of the variability from the first five principal components. The heatmap in Figure 5A shows all normalized tuning curves of all significantly tuned multi-units in our database, arranged by cluster membership. It illustrates the variety of tuning curves in each cluster. Figure 5B shows the 480 mean tuning curve for each cluster. The first and second clusters contained two varieties of

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contralateral dominant tuning, with peaks near -100 μs, and accounted for 41% (n=842/2047) and 30% (n=608/2047) of all multi-units, respectively. Together these two contralaterally tuned clusters comprise the large majority of ITD multi-units, as would be expected in light of previous findings (Hancock et al. 2013; Tillein et al. 2016). The primary difference between these two clusters is that the tuning curves in the cluster 1 (dark blue in Fig. 5B and C) exhibit a second, slightly smaller peak for ipsilateral ITDs at +120 μs, and they may therefore be described as “trough” shaped, unlike tuning curves in cluster 2 (light blue in Fig. 5B and C) which exhibit only a single substantial peak for contralateral ITDs at -100 μs and are thus considered “contralateral” in shape. However, both clusters 1 and 2 are clearly predominantly contralaterally tuned. The third largest cluster contained mostly multi-units which gave strongest responses for ITDs near zero, “central” tuning, and comprised 25% (n=517/2047) of the significantly tuned multi-units. The fourth cluster comprised only 4% (n=80/2047) of multi-units, and these peaked for ipsilateral ITDs at 100 μs and are thus considered as ”ipsilaterally” tuned.

Next, we asked whether each of the four clusters of tuning curves (“trough”, “contralateral”, “central”, and “ipsilateral”) was equally represented in the neonatally deafened and hearing experienced cohorts. Figure 5C shows the distribution for each of the four clusters found in 5A and B for each animal. Here we see a fair amount of individual variability. However, we can still appreciate some general trends. In the hearing experienced rats, the majority of units belonged to either the “trough” shaped cluster 1 (768/1081=71%) or the “contralateral” cluster 2 (231/1081=21.4%), giving a total of 92.4% of multi-units with predominantly contralateral tuning. Central or ipsilateral tuning were rare in multi-units from hearing experienced animals, representing only 3% and 5% of our sample, respectively. In contrast, the neonatally deafened animals were found to have far fewer contralateral peak type units, at only 46.6% (74/966=7.7% from “trough” cluster 1 and 377/966=39% from “contralateral” cluster 2) with the exception of ND4. Instead, in neonatally deafened rats far more multi-units (50% of the total) were found with peak tuning near the midline (“central” cluster 3). Ipsilateral tuning (cluster 4) was rare in the neonatally deafened

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samples, at 3%, similar to that in the hearing experienced cohort. The distributions of the four different clusters per animal are shown as percentages in Table 3.

Clusters	ND1	ND2	ND3	ND4	HE1	HE2	HE3	HE4
1	22.85	0	5.10	0	79.40	0	62.09	94.56
2	46.01	0	32.94	97.7	18.21	85.83	17.33	5.44
3	23.97	100	58.43	2.30	1.20	14.17	3.25	0
4	7.12	0	3.53	0	1.19	0	17.33	0

Table 3: Distribution of clusters for each animal shown as percentages of the total number of units per animal.

The difference in “predominantly contralateral” tuning in the two cohorts is thus quite large (92.4% in HE vs only 46.6% in ND, a 45.8% difference) but the results from the ND cohort are also quite heterogeneous (compare animal ND2 and animal ND4 in Fig. 5C) which makes assessing the statistical significance of this difference with conventional methods difficult. One would have to take into account the “nesting” of individual variability into presumed cohort variability. One can, however, run a simple simulation to ask how easily the observed differences between the cohorts can arise from nothing more than a simple random sampling effect. Let us assume that the variability in percentages of contralaterally tuned units seen in Figure 5C is representative of rats in general, irrespective of whether they are ND or NH. Taking values straight from Figure 5C we know that rats can have percentages of contralateral tuning that can take the values 68.9, 0, 38, 97.7, 97.6, 85.8, 79.4 or 100%. It is of course very unlikely that these 8 observed values are the only percentage values that a rat chosen at random could yield, but for simplicity let us assume that these values are representative of rats in general, and that bootstrap samples drawn from these 8 values would yield average contralateral tuning percentages that won’t be too dissimilar from those one might see in new samples that one could obtain in further experiments. Under this assumption, we bootstrapped 1000 “simulated ND” and 1000 “simulated HE” cohorts of 4 animals

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each, by drawing sets of 4 values from the set {0, 38, 79.4, 68.9, 85.8, 97.6, 97.7, 100} for each simulated cohort, with replacement. We then compared the average contralateral tuning percentage seen in each pair of simulated cohorts, and we observed that only ~4.9% of these 1000 pairs of bootstrapped cohorts had absolute differences in mean percentage contralateral tuning that reached or exceeded the 45.8% seen in our original data. This bootstrap test thus just reaches statistical significance, and gives some indication that the large observed difference in contralateral tuning between the two cohorts may be larger than expected by chance, even if we take into account the considerable individual variability.

To further confirm that the trends seen in Figure 5C are robust, we determined the “best ITD” (that is, the ITD value giving the largest response) for each multi-unit, and plotted the distributions in Figure 5D for both the hearing experienced (blue) and the neonatally deafened (salmon-pink) cohorts. Overall, we see that both cohorts demonstrate two peaks. Again we note differences between cohorts: the majority of multi-units recorded in the hearing experienced animals (blue bars) have their maximum responses at contralateral ITDs (-100 to -120 μs), with a much smaller peak for ipsilateral ITDs (+120 μs) which correspond to clusters 1 and 2 in Figure 5B and C. Tuning curves of multi-units recorded from neonatally deafened animals (salmon-pink bars) also often have their maxima at contralateral ITDs with a large portion showing peak response at -100 μs. However, in contrast to the hearing experienced data, these multi-units commonly exhibit maxima just ipsilateral to the midline, with best ITDs between 0 and +20 μs corresponding to cluster 3 in Figure 5B and C. This best ITD distribution arises because the “centrally” tuned responses that form cluster 3 in Figure 5B are not exactly at the midline, but slightly offset toward the ipsilateral ITDs. Overall, we observed that hearing experienced animals showed a clear contralateral dominance for best ITD, while the neonatally deafened cohort showed equal proportions of units with central, or just ipsilateral, and contralateral peak ITDs.

The differences in the distributions of tuning curve types and best ITD illustrated in Figure 5C and D are pronounced. However, assessing the statistical significance of these differences is again

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complicated by the fact that tuning curves of neighboring multi-units cannot be considered independent observations. We therefore opted to perform a highly conservative Kruskal-Wallis test, comparing best ITD averaged over all tuning curves from each of the 79 multi-electrode penetrations in our fine-sampled animals. Thus, each multi-channel electrode penetration contributed only a single best ITD value to this test. The null hypothesis was that the median best ITD would be the same in both the hearing experienced and the neonatally deafened cohort, but the data in Figure 5D suggest that they may be different. Hearing experienced animal tuning curves had a median best ITD at a firmly contralateral -100 μs, while the median best ITD for the neonatally deafened animal tuning curves was just contralateral off the midline, at -20 μs. The Kruskal-Wallis test confirmed that these differences in median were significant with $p = 0.00004$. Note that this test does not allow for “nesting” of penetrations within individual animals, and may therefore overestimate the true level of statistical significance. To the best of our knowledge there is no routinely accepted or widely available method for dealing with nested non-parametric data.

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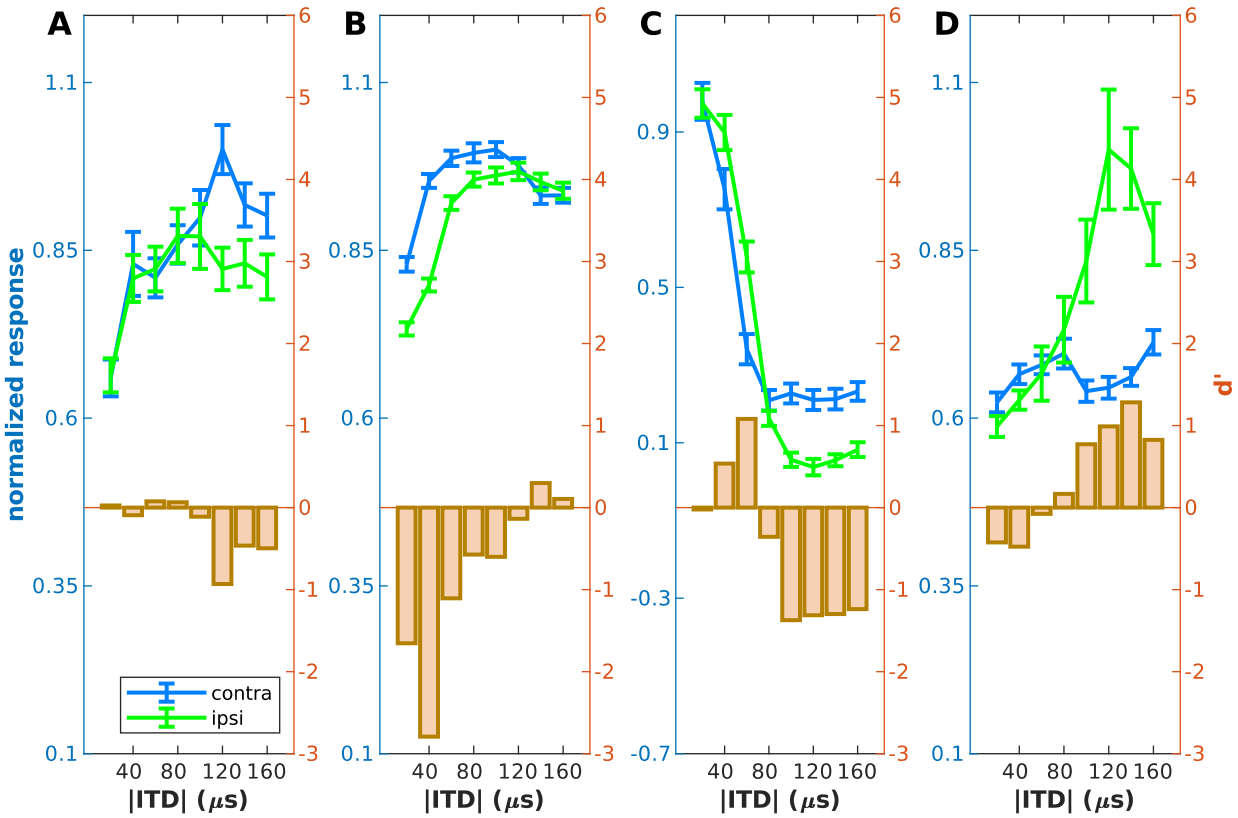

Figure 6: Four examples of multi-units with different tuning curve shapes, plotted alongside their respective *d′* values. Blue y-axis on the left shows the normalized responses, with both the contralateral tuning curve segment (blue) and the ipsilateral segment (green) plotted against increasing absolute ITD. Error bars show the standard error of the mean. The orange y-axis on the right gives the *d′* values plotted as bronze bars below the tuning curves for each of the four examples. Note how absolute *d′* values are large when the distance between the ipsi- and contralateral tuning curve is large relative to the standard error of the mean. *d′* values are positive when the response to the ipsilateral ITD is larger than that to the contralateral ITD and negative for the reverse case.

The substantial differences in tuning curve distributions between neonatally deafened and hearing experienced animals which we have just described raises the question of how these differences might affect an animal's performance in particular types of sound localization tasks. For example, midline tuned multi-units have tuning curves that are fairly symmetric around the midline, and might therefore be expected to be less suitable for signaling whether sound came from the left or right

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than either ipsi- or contralaterally tuned units. At present, our understanding of how the activity of inferior colliculus neurons is read out by thalamic, and ultimately, cortical neurons to control sound localization tasks is very incomplete, and any assessment of the neural coding at the level of the inferior colliculus in order to predict limits of behavioral performance will depend on a numerous assumptions. Nevertheless, it is possible to analyze neural responses using the tools of signal detection theory to compute receiver operating characteristics and sensitivity *d′* indices, which can serve as theoretical upper bounds of the discriminability of pairs of stimuli (Geissler 2003). Here, we opted to evaluate how well the observed neural tuning of each multi-unit might support the performance in an ITD lateralization task by computing *d′* values for the observed distributions of neuronal responses to pairs of contralateral and ipsilateral stimuli for a given ITD. Figure 6 shows responses and derived *d′* values (computed as described in the methods) for four example units, one contralaterally tuned (A), one trough shape (B), one centrally tuned (C), and one ipsilaterally tuned (D). To put the values plotted along the right y-axes in Figure 6 into perspective, remember that *d′* values relate to the percent correct scores that an ideal observer should be able to achieve in a two-alternative forced choice task according to the relationship $percent\ correct = \Phi(d'/\sqrt{2})$, where Φ is the cumulative normal distribution. Also remember that the sign of the *d′* reflects only whether a unit fires more strongly for contra- or ipsilateral stimuli. Normally inferior colliculus units exhibit predominantly contralateral tuning, which our analysis maps on to negative *d′* values, but units with reliable ipsilateral tuning and therefore strongly positive *d′* values could in principal also guide successful lateralization behavior. Some of the analysis below will therefore consider absolute *d'* values (|*d′*|). We remind the reader that a |*d′*| of 1 is equivalent to an upper limit of performance of ~75% correct, while a |*d′*| of 3 is equivalent to an upper limit of ~98% correct. With these values in mind, we note that all of the multi-units shown in Figure 6 should be capable of supporting behavioral performance much above chance level in a two-alternative forced choice ITD lateralization task, but the four multi-units shown differ in the range of ITDs for which they can facilitate lateralization at 75% correct performance, in other words where they have an |*d′*| value of 1 or above. Note also that the trough or central peak tuned units shown in Figure 6B and C, which

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due to the relative symmetry of their tuning curves might be considered less suitable for lateralization tasks, nevertheless are sufficiently left-right asymmetric to yield quite sizable |*d′*| values for certain ITDs.

In Figure 7A we show the distributions of *d′* by the multi-units recorded for each pair of left and right leading ITDs. The distributions for both the neonatally deafened (pink bars) and the hearing experienced (blue bars) cohorts are plotted as overlapping histograms. The distribution of *d′* values for the neonatally deafened group appears to be wider at all ITDs. Additionally, the neonatally deafened group has more positive *d′* values, particularly for ITDs of +/-40 and +/-60 μs and more negative *d′* values for ITDs ≥ +/-80 μs. One question we can address with the data in Figure 7A is: how many multi-units would support "suprathreshold" performance, which we define here as |*d′*|>1, and would therefore be capable of facilitating an above ~75% correct performance by an optimal observer in a two-alternative forced choice lateralization task for a given ITD? These multi-units lie outside the range of $d' \in [-1, 1]$ indicated by the broken lines in Figure 7A. The proportion of these multi-units for each ITD value in each cohort are summarized in Figure 7B (neonatally deafened) and C (hearing experienced)

Figure 7B and C show the proportion of units with an ITD sensitivity large enough to be theoretically capable of supporting an ITD lateralization performance of up to 75%, where d' values were either below -1 (dashed lines) or above +1 (solid lines). Hearing experienced animals showed almost no units with a d' > +1 which would indicate a strong ipsilateral lateralization (Fig. 7C, solid line). However, the number of units with good contralateral encoding (d' < -1) increased sharply after +/-40 μs with a maximum between 100 to 120 μs (Fig. 7C, dashed line). The proportion of multi-units with absolute d' > 1 for neonatally deafened animals (Fig. 7B in gray) is slightly higher than that for hearing experienced animals, both for negative (dashed line) and positive (solid line) d' values. As in the hearing experienced cohort, the neural responses from the neonatally deafened animals also showed a sharp increase in the proportion of units with d' < -1 as ITDs increased above +/-40 μs. If we consider the absolute d' values we see that both cohorts show a

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peak response between 100 -120 µs. These increases in multi-unit proportions with sizable d' values for increasing ITD qualitatively match similar increases in behavioral ITD discrimination abilities previously demonstrated (see Supplementary Fig. 1; Li et al. 2019; Rosskothen-Kuhl et al. 2021).

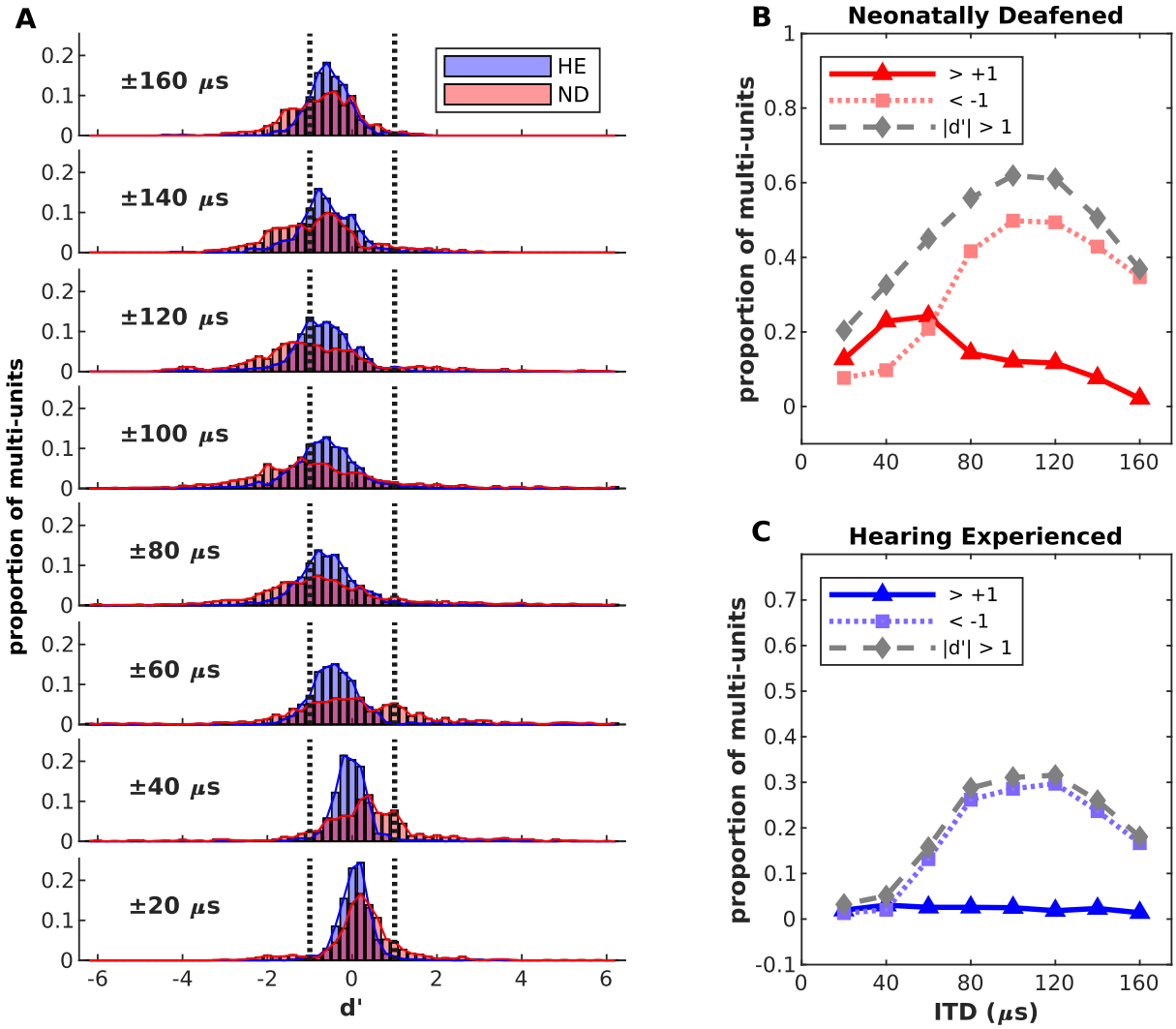

Figure 7: A: Distributions of *d'* values for pairs of ITD values shown on the left. Negative *d'* values indicate stronger contralateral responses, positive *d'* values indicate stronger ipsilateral responses. Distributions for hearing experienced animals are shown in blue, those for neonatally deafened animals in pink. Broken vertical lines highlight d' values of +/-1, equivalent to a discrimination performance of ~75%. B: Proportions of multi-units with *d'* either > +1 (solid lines) or < -1 (dashed lines), as a function of ITD, for the neonatally deafened cohort. The gray dashed line with

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diamond marker shows the sum of these in other words |d'| >1. C: as in B, but for the hearing experienced cohort. HE: hearing experienced animals, ND: neonatally deafened animals.

3.4. Sampling ITDs predominantly outside the physiological range and excluding onset responses resulted in sizable reductions in measured ITD sensitivity

As mentioned above, most previous studies of ITD tuning have sampled a very wide range of ITD values, often several times larger than the animal's physiological range, at fairly coarse intervals. Our results here are unusual in that we found ITD tuning in neonatally deafened CI animals which was no less robust than that seen in hearing experienced controls. Importantly, the +/-160 µs range of ITDs we tested barely extends beyond the animals' physiological range of +/-120 µs (Koka et al. 2008). Within this range, we sampled with a 20 µs step size, which is small enough to resolve behavioral just noticeable differences, which are in the order of ~50 µs (Rosskothen-Kuhl et al. 2021). It seems possible that previous reports of impaired ITD tuning in CI animals could be adversely affected by sub-optimal sampling of ITD values, for example if the range of ITDs sampled is unnaturally large, several times larger than the range of values that the system would have evolved to process, or if the sampling resolution is too coarse relative to known or expected behavioral thresholds.

To investigate that possibility, we recorded inferior colliculus ITD tuning curves from additional four neonatally deafened rats, using the same procedures, but sampling a wider ITD range, from -300 to +300 µs, in coarser, 75 µs step sizes. This "wide and coarse sampling" spans a range of ITDs that corresponds to 250% of the normal physiological range in our animal model, and is more similar to ranges adopted by other authors in previous studies. Figure 8A shows the distribution of mutual information values for the 406 ITD tuning curves recorded with wide and coarse sampling during 27 multi-channel electrode penetrations into the inferior colliculi of the second batch of four neonatally deafened animals. For easy comparison, the mutual information values for the neonatally deafened animals sampled with our tight sampling which was already shown in Figure 4A is also reproduced in Figure 8B. The differences in these distributions are pronounced. Of the coarsely sampled units, only 52.7% (n=214/406) had mutual information values that were significantly above zero, in contrast to the finely sampled units, where 84.8% (966/1139) were significantly ITD sensitive. When including both significant and non-significant mutual information

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values we found that the median mutual information value for the coarsely sampled data was 0.06 bits/response compared to 0.3 bits/response for the fine sampled cohort. Based on a Kruskal-Wallis test, this difference was significant ($p < 0.001$).

If we were to additionally exclude at least the first 15 ms of the response in our dataset, as has been done in a few previous published studies (Hancock et al. 2010; Hancock et al. 2012; Hancock et al. 2013; Chung et al. 2019), then the proportion of multi-units with significant mutual information values, drops from 52.7% to as low as 5% with coarse sampling and from 84.8% to 61% with fine sampling ITDs (data not shown). A similar drop in ITD sensitivity was seen with the finely sampled data from the hearing experienced cohort (data not shown). However, it should be noted that the absence of sustained responses is not too surprising given that these were single pulse stimuli.

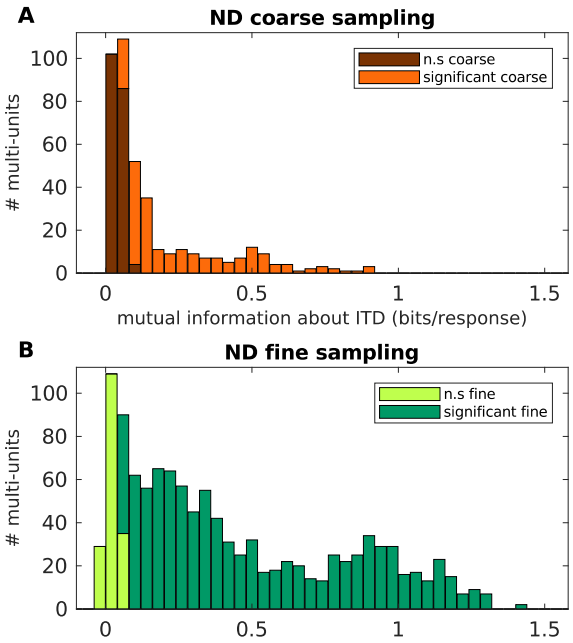

Figure 8: Coarsely and broadly sampled ITD tuning curves recorded in neonatally deafened CI rats yielded lower sensitivity values than ITD tuning curves which finely sample the physiological range. A: Mutual information values for coarsely sampled ITDs (from -300 µs to +300 µs in 75 µs steps). B: Histogram of mutual information values from the cohort with finely sampled ITDs (from -160 µs to +160 µs in 20 µs steps) reproduced from Figure 4A for comparison. Mutual information values which are significantly above zero are shown in orange (A) or dark green (B). ND: neonatally deafened animals, n.s.: non-significant.

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4. Discussion

4.1. Inferior colliculus neurons showed prominent ITD sensitivity even in the absence of hearing experience

In this study we have documented an abundance of ITD tuning in the inferior colliculus of cochlear implanted rats immediately after bilateral cochlear implantation, even in the absence of early auditory experience. ITD sensitivity has been previously reported in early deaf animal models, both in inferior colliculus and in auditory cortex, although it was reportedly weaker than in hearing experienced controls (Hancock et al. 2010; Tillein et al. 2010; Hancock et al. 2013; Chung et al. 2016; Tillein et al. 2016; Vollmer 2018; Chung et al. 2019). Before we turn our attention to the differences between our findings and those reported by others, we must note that there is an important agreement among all studies of ITD sensitivity in early deaf CI animals to date: none of these studies has yet observed a reduction in ITD sensitivity compared to normal which would be large enough to adequately explain the severe lack of behavioral ITD sensitivity observed in early deaf human patients with bilateral CIs (van Hoesel 2004; Grieco-Calub and Litovsky 2010; Litovsky 2010; van Hoesel 2012; Kerber and Seeber 2012; Laback et al. 2015; Ehlers et al. 2017).

In contrast to the above mentioned studies on deaf animal models, our results are unusual in that we did not find any marked decrease in the quality of ITD tuning in our neonatally deafened animals compared to hearing experienced controls. Indeed, the ITD sensitivity in neonatally deafened CI animals was comparable to hearing experienced CI animals (Fig. 4A, B). There are many possible reasons for this possible discrepancy. One of course, is species differences, given that we used rats while other previous studies have used predominantly cats or rabbits. However, as shown in the context of Figure 8, the very robust ITD tuning in neonatally deafened animals is only apparent if the ITDs sampled focus on the physiological range, and if onset responses are included in the analysis. Other previous studies (e.g. Hancock et al. (2012); Hancock et al. (2013))

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may therefore have missed some ITD sensitivity in their early deaf CI animals due to their use of coarse and wide ITD sampling and exclusion of onset responses. However, it should be noted that whether or not the effects of coarse and wide ITD sampling are hearing experience dependent is unknown. Never the less what may have motivated the wide and coarse ITD sampling choices made in previous studies of ITD sensitivity in CI animals? Unfortunately, these articles do not describe how the authors chose the ITD values tested, but it seems very likely that they simply followed the example set by classic studies on ITD sensitivity in the brainstem and midbrain in response to acoustic, often pure tone stimulation. Indeed, most previous studies of ITD coding under acoustic stimulation have used ranges of ITD values that extend far beyond the physiological range (Yin and Chan 1990; McAlpine et al. 1998; Brand et al. 2002; Yin 2002). With acoustic stimuli such a wide range of ITDs can be useful. For example it can reveal periodic ITD tuning curve shapes at periods, which reflect a unit's characteristic frequency. This, in turn can hint at the nature of ITD detection circuits in the brainstem, revealing a "cross-correlator-like" operation, in which periodic inputs from the cochlear filters produce periodic outputs (Schnupp and Carr 2009). Thus, in the context of studies with acoustic stimuli, which are interested in possible underlying neural mechanisms, sampling unnaturally large ITD ranges can be revealing. However, CI stimulation bypasses the cochlea's mechanical filters. There is no filter ringing which would induce periodic auditory nerve responses, and obvious periodicities in midbrain ITD tuning curves, which are so common with acoustic stimuli, are neither expected nor observed under CI stimulation. Furthermore, in studies of prosthetic hearing, the focus is often more on likely capabilities rather than underlying mechanisms. Our objective here was to assess the likely capabilities of the binaural system after neonatal deafening. Our exclusion of unnaturally large ITDs in favor of a fine-grained focus on the physiological range is well motivated, even if it makes it difficult to compare our results directly with those of other previous studies which did not prioritize the use of ITDs within the physiological range and step sizes which are small enough to resolve behavioral just noticeable differences,

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Another important difference is that our quantification of response strength included onset responses, while several other studies (Smith and Delgutte 2007; Hancock et al. 2010; Hancock et al. 2012; Hancock et al. 2013; Chung et al. 2019) excluded them. In our analysis we used a response window from 2.8 – 40 ms post-stimulus onset. Our study focused on optimizing the delivery of ITDs and as such we looked at the most salient aspect of the ITD cue response namely the onset ITD responses (Brown and Stecker 2010; Greenberg et al. 2017). To us, including onsets in the analysis seems well motivated, given that Brown and Stecker (2010) and others have shown that the onset of stimuli dominates the perception of both ITDs and ILDs in normal hearing human listeners, and that physiological studies have ascertained that stimuli with sharp onsets yield better ITD sensitivity (Greenberg et al. 2017). Indeed, many studies of the so-called "precedence effect" have documented the dominance of sound onset in spatial hearing, and highlighted the usefulness of strong onset weighting in reverberant acoustic environments, where only the earliest part of a sound stimulus can be expected to be uncontaminated by confounds generated by strong echoes created by indirect sound reflected off nearby surfaces (Litovsky et al. 1999; Brown et al. 2015a,b). Studies which analyzed exclusively or predominantly the sustained part of neural responses to ongoing stimuli therefore exclude a very important portion of the neural response, and are bound to underestimate the "true" ITD sensitivity of the neurons studied. In fact, when we excluded onsets from our analysis, the proportion of multi-units exhibiting statistically significant ITD sensitivity in the group of neonatally deafened animals tested with the "wide and coarse" stimulus set dropped dramatically from 52.7% to only 5% and with fine sampling from 84.8% to 61%. However, it is not surprising that we see few units with sustained ITD responses as our stimuli were not designed to deliver such cues unlike the previous studies. Additionally, at least with the fine sampling data, a similar drop in the proportion of ITD sensitive units was seen for the hearing experience animals (data not shown) with the exclusion of onset responses. Nevertheless, these results indicate that details in the choice of stimulus parameters range as well as whether analysis time windows focus on onset or sustained responses can have dramatic effects on the quality of the ITD tuning observed. In our study, we chose parameters which we believe to be well

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motivated from a perspective of ecological validity, with ITDs mostly confined to the physiological range, and onset responses included in the analysis, given the well known onset-bias of binaural processing. In summary, we believe that methodological differences may be chiefly responsible for the fact that we did not observe the reduction of ITD sensitivity in neonatally deafened animals that has been previously described by others.

4.2. Increased neuronal excitability in the absence of hearing experience

Multi-units from our neonatally deafened rats showed appreciably stronger responses, as well as higher mutual information values for ITD tuning, compared to the multi-units from hearing experienced rats (Fig. 4). eABR thresholds and stimulus amplitudes were similar in the two groups, so the increased activity is likely due to biological factors. This is reminiscent of observations by (Hancock et al. 2010; Hancock et al. 2013) that spontaneous activity is increased in long-term deafened or congenitally deaf cats when compared to acutely deafened animals. Homeostatic plasticity may limit the strength of responses to sensory inputs in hearing experienced animals, but not in neonatally deafened animals. Neonatally deafened animals would then exhibit a form of hypersensitivity when they are supplied with CI stimulation for the first time in these experiments. Support for this hypothesis comes from reports showing that inhibitory interactions weaken and inhibitory synaptic strengths decrease in the deafened auditory system (Bledsoe et al. 1995; Abbott et al. 1999). Similarly, Tirko and Ryugo (2012) have shown that numbers of inhibitory axosomatic terminals in the medial superior olive (MSO) were substantially reduced in deafened animals, and Vale et al. (2003) and Vale and Sanes (2002) found that the inhibitory synaptic strength in the central inferior colliculus of gerbils declines after deafening, while excitatory post-synaptic currents increase. Auditory cortical excitability too becomes stronger following hearing loss, with increased excitatory post-synaptic potential amplitudes as well as substantially less GABAergic inhibitory activity (Kotak 2005). In a similar vein, some of our earlier studies have observed significantly

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larger numbers of activated inferior colliculus neurons in neonatally deafened rats compared to hearing experienced controls after identical schedules of CI stimulation (Rosskothen-Kuhl and Illing 2012; Rauch et al. 2016; Rosskothen-Kuhl et al. 2018). In addition, CI stimulation of neonatally deafened, but not hearing experienced rats has been shown to modulate the inhibitory network of the inferior colliculus resulting in an up-regulation of inhibitory markers, such as glutamic acid decarboxylase (GAD) GAD65 and GAD67 (Rosskothen-Kuhl et al. 2018). We therefore expect increased excitation and reduced inhibition along the auditory pathway of a deafened animal during initial electrical intracochlear stimulation in an acute experiment, as this should lead to stronger responses and perhaps also to a higher “signal-to-noise-ratio” or “signal-to-total-variance-ratio” in the encoding of stimulus parameters, which may explain the high levels of mutual information between stimulus parameter and response we observed in our neonatally deafened animals (Fig. 4).

4.3. Substantial differences in tuning curve shapes between hearing experienced and inexperienced animals

In Figure 5 we documented apparent differences in tuning curve shape distributions between neonatally deafened and hearing experienced cohorts. These differences were large and appear to be statistically robust as suggested by a highly significant Kruskal-Wallis test. However, the cohort sizes were relatively small, individual differences were quite marked (see Figure 5C), and one cannot completely exclude the possibility of electrophonic responses in the hearing experienced animals generating some sort of confound, even if it is hard to see how that would work. We therefore do not wish to exaggerate the statistical reliability of the observed differences in tuning curve distributions. However, differences in tuning curve shapes between early deafened and hearing experienced cohorts have been reported in previous studies (Hancock et al. 2012; Hancock et al. 2013). Additionally, these studies point to experience dependent mechanisms that appear capable of altering ITD tuning curve shapes in the auditory brainstem. Furthermore, our

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finding of dominant contralateral tuning in our hearing experienced cohort was also observed in previous studies which investigated ITD sensitivity in the inferior colliculus of other animal models under electric stimulation (McAlpine and Palmer 2002; Smith and Delgutte 2007; Hancock et al. 2010; Hancock et al. 2012; Hancock et al. 2013). These facts make it plausible to assume that there may be systematic differences in the distributions of ITD tuning curves observed in hearing experienced or inexperienced animals, respectively, and thus the group differences we reported here are likely robust in spite of individual variability or a sampling bias.

Much previous work has classified ITD tuning curves into four main types: sigmoid, biphasic, trough, and peak/multi-peak shaped, based on how well the tuning curves correlated with predefined canonical shapes, such as “peak”, “trough”, “biphasic” or “sigmoid” (Smith and Delgutte 2007; Hancock et al. 2010; Tillein et al. 2010; Hancock et al. 2012; Hancock et al. 2013; Chung et al. 2016; Tillein et al. 2016; Vollmer 2018; Chung et al. 2019). Some of these studies have documented differences in the proportions of tuning curves in each of these classes between early deafened and hearing experienced animals. We decided not to assume predefined tuning curve shapes, in part because we sampled a narrower, physiologically relevant range of ITDs much more densely, which is bound to affect the range of shapes observed, and in part because we generally favor data-driven approaches with minimal prior assumptions. Nevertheless, the clusters we observed do resemble the “peak”, “trough” and “biphasic” shapes used by others. A direct comparison of proportions of observed tuning curve “types” between studies is hindered by numerous methodological details, including the very different sets of ITDs tested. Nevertheless, we can observe clear parallels. For example, several studies have reported greatest slopes near ITDs of zero (McAlpine et al. 2001; Brand et al. 2002; Shackleton et al. 2003; Hancock and Delgutte 2004). Our best ITD distributions, for trough or central clusters (Fig. 5B and D) are in line with these previous observations, and are comparable to those seen in Figure 4A of Hancock et al. (2013). Thus, even if we cannot make precise quantitative comparisons, we nevertheless note

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clear qualitative agreement in the types of tuning shapes seen, and in the fact that proportions of shapes seen may differ depending on hearing experience status.

So what might drive such experience dependent differences in ITD tuning curve shapes? ITD sensitivity observed in the inferior colliculus is usually thought to arise first in the superior olivary complex, particularly the MSO, but particularly in animals with relatively high frequency hearing, such as rats, envelope ITD coding through the lateral superior olive (LSO) is also likely to make important contributions (Joris and Yin 1995). The development of ITD sensitivity in the MSO has so far been studied in much greater detail. A number of studies have demonstrated that inhibitory inputs to the MSO play a major role in shaping ITD tuning curves (Brand et al. 2002; Pecka et al. 2008; Leibold 2010; Myoga et al. 2014; Beiderbeck et al. 2018), and Kapfer et al. (2002) have shown that inhibitory glycinergic inputs to the MSO undergo postnatal developmental refinement. Beiderbeck et al. (2018) used models to explore how the timing of the inhibition can suppress or facilitate neural spiking and confirmed their simulated findings *in vitro.* Pecka et al. (2008) used glycinergic antagonists to demonstrate the importance of inhibitory inputs to the MSO in shaping ITD tuning curves, and a modeling study by Leibold (2010) illustrated how ITD tuning curves can be shaped by the balance of inhibitory and excitatory inputs, and these in turn appear to be amenable to modification through experience dependent plasticity (Seidl and Grothe 2005). Similar mechanisms may well occur in ITD processing pathways of the LSO, but they have not yet been investigated. Nevertheless, ITD processing pathways can clearly be refined by experience, but that does not imply that binaural neurons lacking early experience cannot be highly ITD sensitive. We therefore think it likely that the differences in tuning curve shapes observed between our hearing experienced and neonatally deafened animals reflect differences in the amount and nature of experience dependent plasticity. It would be interesting to know whether hearing experience in adulthood, through CIs, can change tuning curve shapes in neonatally deafened animals, or whether it is developmentally regulated. This may be possible, given that there is some

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evidence of adult plasticity in binaural pathways, for example in response to a loss of stimulation (Vale and Sanes 2002) or a supply of stimulation through CIs (Rosskothen-Kuhl et al. 2018).

5. Conclusions

Our multi-unit recordings from the inferior colliculus of four neonatally deafened and four hearing experienced rats, all of which were acutely implanted with bilateral CIs as young adults, pointed to the presence of large amounts of innate ITD sensitivity even in the absence of early auditory experience when sampled appropriately. Even though the ITD tuning appeared somewhat abnormal, with fewer contralaterally tuned multi-units in the neonatally deafened compared with the hearing experienced animals. However, our mutual information and *d′* analyses showed that the ITD tuning in neonatally deafened animals is nevertheless highly informative about ITD values in the physiological range, and they should therefore be able to support accurate ITD discrimination in spatial hearing tasks. To what extent these findings translate to human patients remains to be seen, but they do suggest that early deaf CI patients fitted with binaural CIs may not be fundamentally ITD insensitive, poor psychometric results in previous studies notwithstanding. Perhaps good functional ITD sensitivity could be elicited in early deaf humans if they are supplied with adequate stimulation and training following CI insertion.

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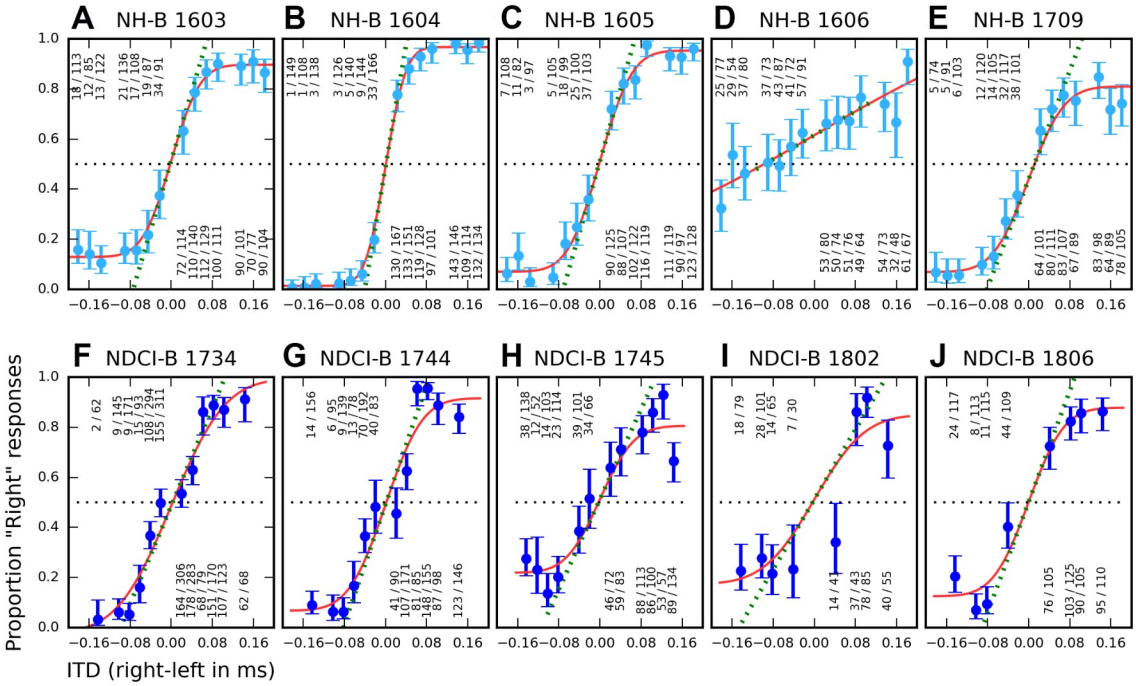
Supplementary Figure



Supplementary Figure 1: ITD psychometric curves of normal hearing acoustically stimulated (NH-B, A-E) and neonatally deafened CI-stimulated rats (NDCI-B, F-J). Panel titles show corresponding animal IDs. Y-axis: proportion of responses to the right-hand side. X-axis: Stimulus ITD in ms, with negative values indicating left ear leading. Blue dots: observed proportions of “right” responses for the stimulus ITD given by the x-coordinate. Number fractions shown above or below each dot indicate the absolute number of trials and “right” responses for corresponding ITDs. Blue error bars show Wilson score 95% confidence intervals for the underlying proportion “right” judgments. Red lines show sigmoid psychometric curves fitted to the blue data using maximum likelihood. Green dashed lines show slopes of psychometric curves at x=0. Slopes serve to quantify the behavioral sensitivity of the animal to ITD. The data and figure shown here are originally from our previous study (Rosskothen-Kuhl et al. 2021).

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