Unilateral conductive hearing loss causes impaired auditory information processing in neurons in the central auditory system

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Temporary conductive hearing loss (CHL) during development and in adults can lead to hearing impairments that persist beyond the CHL. Despite decades of studies, there is little consensus on the mechanisms responsible. Here we introduced 6 weeks of unilateral CHL to adult chinchillas via a foam earplug. Single-unit recordings from inferior colliculus (IC) neurons indicated that the CHL caused a decrease in the efficacy of inhibitory input to the IC contralateral to the earplug and an increase of inhibitory input ipsilateral to the earplug. The changes were seen after removal of the CHL. Sensitivity to interaural-level-difference (ILD) cues to location in IC neurons was shifted by ~10 dB relative to controls. In both ICs, the direction of the shift was consistent with a compensation of the altered ILDs due to the CHL. IC neurons responses carried ~33% less information (mutual information) about ILDs after CHL than normals. Experiments examining cochlear anatomy and peripheral evoked responses confirmed that the results did not arise from damage to the periphery. The CHL-induced shifts of ILD sensitivity suggest a compensatory form of plasticity occurring by at least the level of the IC. The neurons were also impaired in their abilities to encode information about the spatial attributes of sound. How these physiological changes may lead to impaired hearing will be discussed.

INTRODUCTION

Conductive hearing loss (CHL) during development can change auditory system structure and function (see reviews by Moore and King, 2004; Tollin, 2010; Whitton and Polley, 2011). Early life exposure to CHL, particularly unilateral, can lead to impairments in binaural hearing even after resolution of the CHL and hearing sensitivity in both ears returns to normal. The persistently-impaired binaural hearing often recovers, but this can take months or years. During recovery, a child may present as audiologically normal, yet speech perception in noisy, reverberant environments may continue to be compromised. As language is often learned in such environments, these impairments may contribute to deficits in language acquisition. Decades of studies of the neural, anatomical and behavioral consequences of experimentally-induced CHL in animal models have revealed effects related to the timing of onset, the duration, and the severity of the deprivation. Regarding neural

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processing, these studies have generally demonstrated how only the most basic of neural response properties are altered by early CHL. Yet the persistent binaural behavioral deficits in humans have generally defied explanation based simply on these basic response properties. Currently it is not known whether or how CHL alters the *neural information carrying* capabilities of the auditory system. Towards this goal, we use the novel framework of information theory (Dayan and Abbott, 2001) to investigate how CHL alters information processing in the central nucleus of the inferior colliculus (ICC). Similar persistent impairments in binaural hearing have been reported in human adults that had experienced chronic CHL. Thus, to begin this new line of inquiry, we examine how neural information processing is altered when a CHL is induced in *adult* animals.

METHODS

Eleven young (~P70) adult chinchillas were used for the deprivation experiments while 19 normal-hearing animals were used for control data. Following the method of Lupo *et al.* (2011) a small foam earplug (AO Safety, Indianapolis, IN, USA) was cut to fit snugly into the external ear canal of the animal and was then inserted into the left ear canal for 6 weeks.

Cochlear microphonic (CM) recordings

Animals were anesthetized and prepared for electrophysiology as described by Jones *et al.* (2011). Briefly, a hole (2-3 mm diameter) was made in each bulla through which electrodes were placed on the round windows and fixed in place with dental acrylic, resealing the bullae. The CM was differentially amplified, filtered, and verified by oscilloscope. To quantify the magnitude of the CHL due to the earplug, free-field CM (and compound action potential, CAP) measurements were taken for both the left (plugged) and right (normal) ears for two different conditions: with the earplug in place (left ear) and after the earplug was removed. Stimuli consisted of 10-ms sinusoids (2.5-ms rise/fall, 5-ms plateau) with octave steps from 0.25-20 kHz. Each stimulus was presented at least 25 times with a 40-ms interstimulus period.

Electrophysiological methods

Single unit, extracellular responses were recorded from neurons in the ICC. All recordings in the group of animals with CHL were performed the same day as the earplug removal. Frequency-intensity response areas were measured with tone pips to estimate the characteristic frequency (CF) and threshold. Neuronal ILD sensitivity was examined using 50 repetitions of 50-ms duration CF tones by holding the signal level to the contralateral ear (~20 dB re: threshold) constant and varying the level in 5-dB steps at the ipsilateral ear from at least 25 dB below to 25 dB above ipsilateral threshold. The rate vs. ILD for each neuron was fitted with a 4-parameter sigmoid, $\text{rate(ILD)} = y_0 + \alpha/(1-\exp(-(\text{ILD}-\text{ILD}_0)/\beta))$; before fitting, the data were normalized to the maximum rate. The fits described the data for all neurons ($R > 0.9$). The fit parameters were used for analysis; half-max ILD is the ILD at 50% of the maximal rate, rate-ILD slope (spikes/s/dB, not normalized) was computed at half-max ILD, and ILD dynamic range was defined between 90-10% of max rate.

Neural information analysis – Mutual information computation

The mutual information (MI) is a measure of the strength of the association between two random variables, such as a spike count, *r*, and a given stimulus, *S* (Dayan and Abbott, 2001). MI is given by

$$MI(r,S) = \sum_i \sum_j p(S_j) p\langle r_i \mid S_j \rangle \log_2 \left[\frac{p\langle r_i \mid S_j \rangle}{p(r_i)} \right] \quad \text{(Eq. 1)}$$

where $p(S_j)$ is the probability that the stimulus (S) had a particular value [S values (i.e., ILDs) were presented with equal probability], $p(r_i)$ is the probability that the count was r_i at any value of S, and $p(r_i|S_j)$ is the probability that the count was r_i when the stimulus was S_j. Intuitively, MI will be high when the count variability is larger when computed across different stimuli than the variability computed within single presentations of a particular stimulus. The MI represents the upper bound on the information that even the best 'decoder' could represent. Thus, if CHL changes the information carrying capacity then the MI will capture and quantify it.

RESULTS

Conductive hearing loss due to earplug does not alter periphery

To quantify the CHL caused by the earplug, as well as assay the function of the peripheral auditory system, sound-evoked CM responses were measured while the earplug was still in place and also immediately after earplug removal (see Lupo *et al.*, 2011 and Thornton *et al.*, 2012; 2013 for detailed methods). CM data from the right (unplugged) ear was used as a control; unilateral CHL does not cause residual deficits in the normal-hearing ear (Larsen *et al.*, 2010) and the present data is consistent with this finding. The CHL was ~10-15 dB for frequencies < 4 kHz increasing to ~30 dB > 4 kHz consistent with Lupo *et al.* (2011); the CHL with earplugs was qualitatively similar to CHL due to experimental middle-ear effusion in chinchilla and CHL in children due to effusion (Thornton *et al.*, 2012; 2013). With earplugs, the mean CM thresholds across frequencies and animals were 46.2 ± 7.1 dB. After removal of the earplug, CM thresholds were reduced to 30.7 ± 8.3 dB. Thus, the plug produced an across-frequency attenuation of 15.5 dB. A two-way repeated-measures ANOVA revealed that there was a significant decrease in attenuation after the earplug was removed ($F_{1,10} = 103.3$, $p < 0.0001$). There was no significant difference between the CM thresholds in the control ear and thresholds in the experimental ear after the earplug was removed ($F_{1,15} = 3.71$, $p = 0.073$). The return of CM thresholds to normal levels after earplug removal indicates that the hearing loss induced by the plug was reversible, a finding reinforced by normal amplitudes and thresholds of the CAPs (not shown). Cochlear surface preparations revealed that the integrity of the cochlea was normal and that earplugging did not cause hair-cell death or other cochlear abnormalities. These data indicate that the earplug-induced CHL produced a reversible hearing loss without damaging the periphery.

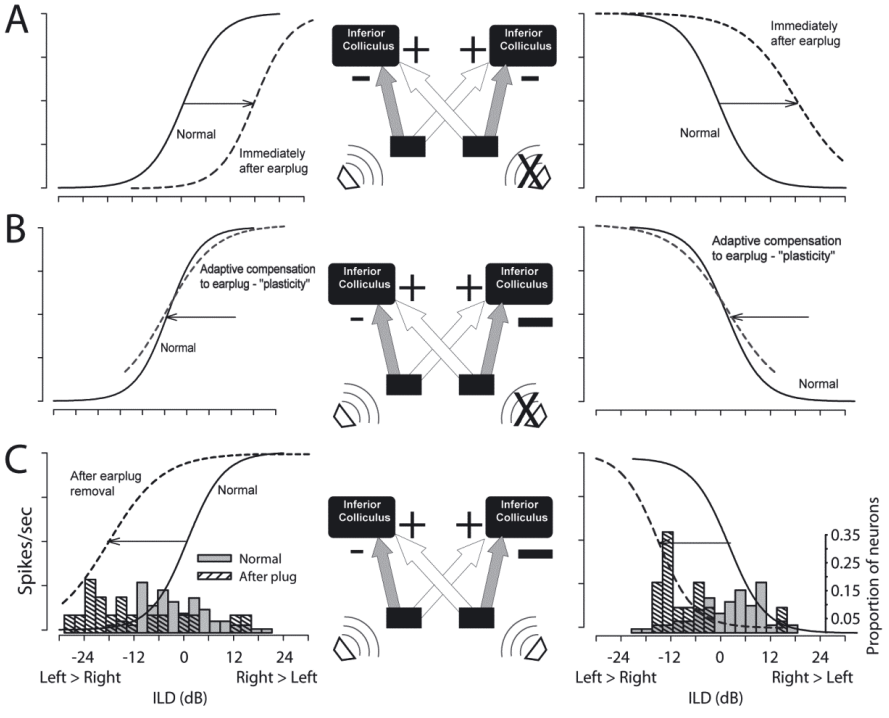

Fig. 1: Hypothesized changes due to CHL (right ear, 'X') in circuit function and the sensitivity to ILDs in the left (contra) and right (ipsi) ICC. The simplified circuit shows ipsi inhibitory ('−') and contra excitatory ('+') inputs to the ICC (strengths indicated by sizes of the symbols). **A**: Normal hearing (solid lines). Neural ILD coding shifts to the right due to CHL (dashed). **B**: Shifts if circuit plasticity has compensated for CHL. **C**: Upon CHL removal, ILD coding shifts left due to the CHL-induced altered circuitry. Histograms: empirical half-max ILDs form IC neurons in normals (n = 31) and after plug removal (n = 31).

Neural coding of interaural-level-difference cues is altered by unilateral CHL

Half-max ILD values were compared between normal animals and animals that received a unilateral earplug as adults. A shift in half-max ILD indicates a shift in the entire rate-ILD curve, signifying that that specific neuron encodes a different range of ILDs. For normal animals, the mean half-max ILD was 1.9 ± 8.3 dB (n = 31 units), with a median value of 0.94 dB and a range of from −20.6 to 17.1 dB (Fig. 1C, left and right panels, grey bars). For neurons in the ICC contralateral to the ear with CHL the mean half-max ILD value was shifted to −10.26 ± 12.2 dB (n = 19

neurons) with a median value of −14.1 dB. The overall range of ILDs encoded by these neurons was shifted toward negative ILDs, ranging from −28.4 to 15.0 dB (Fig. 1C, left panel, gray hatched bars). Relative to controls, the CHL produced an effective shift in ILD coding of 12.2 dB for neurons contralateral to the CHL. An unpaired *t*-test indicated a significant difference in half-max ILDs between normal and earplugged neurons [$t_{(44)} = 3.85$, $p = 0.0004$]. Similarly, for neurons ipsi-lateral to the CHL, the half-max ILDs were shifted to −6.52 ± 8.5 dB (median: −9.4 dB), which was significantly different than controls [$t_{(37)} = 2.92$, $p = 0.006$]. The CHL produced an effective shift in ILD coding of 8.4 dB. The ILD dynamic range of the rate-ILD curves was also impacted by CHL. The mean dynamic range in normals was 26.1 ± 10.1 dB (median: 25.7 dB). For neurons in the ICC contralateral to the CHL, the mean dynamic range was 19.4 ± 9.8 dB (median: 17.6 dB), significantly lower than controls [$t_{(44)} = 2.24$, $p = 0.031$]. Similarly, for neurons ipsilateral to the CHL, the mean dynamic range was reduced to 17.1 dB, significantly different from controls [$t_{(37)} = 2.56$, $p = 0.016$]. Finally, CHL altered the modulation of discharge rate due to ILD. In normals, over the range of ILDs tested the rate was modulated re:

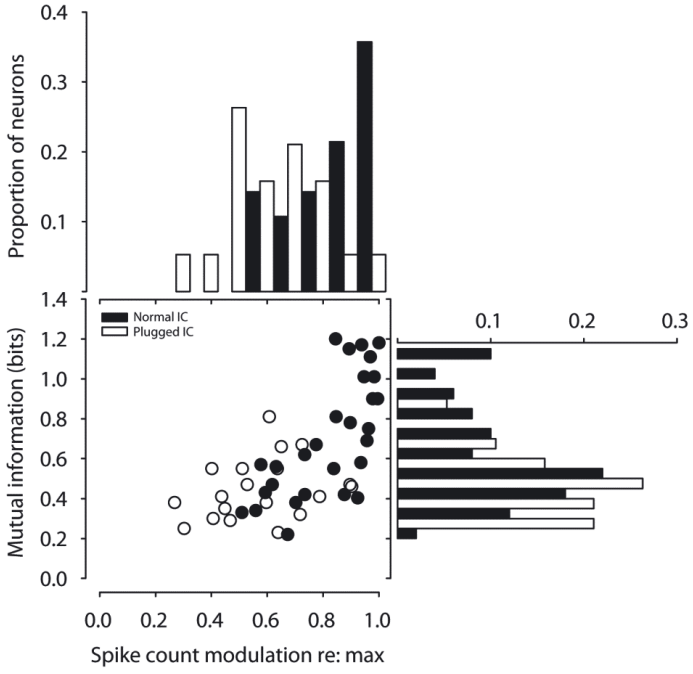

Fig. 2: Mutual information between spike count and ILD in ICC neurons in normal-hearing (black) adults and after 6 weeks of unilateral CHL (grey) is plotted as a function of the spike count modulation by ILD (re: max count).

max rate by 82 ± 16%. Neurons contralateral to the CHL were modulated by 58 ± 18%, significantly less than controls [$t_{(44)}$ = 4.9, p < 0.0001], while neurons ipsilateral to the CHL were modulated by 76 ± 13%, which was not significantly different than controls [$t_{(37)}$ = 1.13, p = 0.26]. Neurons ipsilateral to the CHL were significantly more modulated by ILD than neurons contralateral to the CHL [$t_{(29)}$ = 3.03, p = 0.005]. The reduction in overall rate-modulation by ILD for neurons contralateral to the CHL was due to an increase in the minimum rate at ILDs that should cause inhibition, which is consistent with an overall reduction in inhibition.

IC responses carry less information regarding ILD cues following CHL

Figure 2 shows mutual information computed for 31 neurons from normal animals (black bars, symbols) and for 19 neurons measured in the ICC contralateral to the CHL (white bars and symbols). The mean MI was significantly reduced [$t_{(48)}$ = 3.38, p = 0.001] after CHL from 0.7 ± 0.29 (median: 0.64) bits for normals to 0.44 ± 0.15 (median: 0.41) bits. The responses of ICC neurons thus carried ~37% less mutual information regarding ILD cues following a unilateral CHL as compared to normal controls. Several factors were examined to account for the reduction in information. For neurons contralateral to the CHL, reduction in the information-carrying capacity was consistent with the significant reduction in the amount by which ILD modulated the spike count relative to the max count revealed in the earlier section. This is consistent with an effective reduction in inhibition due to the CHL.

DISCUSSION

Altered inputs to the auditory system can result in anatomical, physiological, and behavioural changes that persist beyond the hearing impairment (reviewed by Moore and King, 2004; Tollin, 2010; Whitton and Polley, 2011). The majority of evidence for CHL-induced plasticity in the auditory system comes from developmental studies in humans and animals. However, studies in adult humans and animals have also suggested that CHL can drive plasticity and that subjects can adapt to altered auditory inputs particularly via behavioural training paradigms. The data presented here suggest a compensatory mechanism for plasticity by at least the level of the inferior colliculus as well as altered information processing. Figure 1 illustrates our general hypothesis regarding compensatory changes in the ascending circuitry to the IC in response to a unilateral CHL. In normal-hearing circuitry (Fig. 1A, solid lines), spike rate is modulated by ILD sigmoidally with maximum responses for ILDs favouring the excitatory contralateral ear and reduced responses for ILDs favouring the inhibitory ipsilateral ear. Immediately after introduction of a CHL (in this case, earplug insertion), the rate-ILD curves would shift toward the right simply because the acoustical input from the contralateral ear has been attenuated (i.e., less effective excitatory input). This is represented by the dashed lines in Fig. 1A.

Figure 1B illustrates the hypothesized circuit changes that would occur if mechanisms were to compensate for the altered sound localization cues due to CHL (see Lupo *et al.*, 2011 and Thornton *et al.*, 2012; 2013). Compensatory mechanisms would work to shift the rate-ILD curves back towards normal (dashed line

overlapping normal curves). To achieve this kind of adaptive compensation in the ICC contralateral to the CHL, the strength (or gain) of inhibitory input from the ipsilateral normal-hearing ear (left side in example) is hypothesized to be reduced and/or the strength (or gain) of the excitation from the contralateral CHL-ear increased; the size of the '+' and '−' symbols have been adjusted in Fig. 1B to illustrate this change. After removing the CHL, the effective changes to the ILD-coding pathways to the ICC can be revealed. If the circuit had been altered as in Fig. 1C, then after CHL removal the rate-ILD curves are hypothesized to shift toward the left (Fig. 1C, black dashed line, left column), demonstrating a reduced ipsilateral inhibitory (and/or increased contralateral excitatory) response when compared to normal. Our data is consistent with this hypothesis.

A similar compensatory response is expected in ICC neurons that are ipsilateral to the CHL. Immediately after introduction of a CHL, there will be an effective reduction in the strength of inhibition to the ICC ipsilateral to the CHL simply due to the attenuation of sound (Fig. 1A, dashed line, right column). If adaptive compensation occurs, the strength of excitatory contralateral inputs will be reduced in order to match the reduced inhibitory inputs and/or an increase in the strength of the ipsilateral inhibitory input to match the normal contralateral excitation. These changes would effectively shift the rate-ILD curves back to normal with the CHL in place (Fig. 1B, dashed line, right column). Immediately after CHL removal, an overall large inhibitory response would remain, causing the rate-ILD curves to shift to the left of normal (Fig. 1C, dashed line, right column). The results are also in agreement with this compensatory model of plasticity.

The present results disagree with previous physiological results in the ICC of animals with unilateral CHL (summarized by Moore and King, 2004; Tollin, 2010; Whitton and Polley, 2011). Prior studies in rats demonstrated that CHL persistently reduced the effectiveness of inputs to the two ICCs from the ear with the CHL, a finding that produces data consistent with illustrations in Fig. 1A as opposed to Fig. 1C. One possible reason for this may be that the experiments in the current study were performed in the chinchilla which is a precocious species (Jones *et al.*, 2011) that also has good low-frequency hearing. Additionally, the results of the prior studies could potentially be due to an altered periphery due to the CHL, such as a residual CHL even after its removal, which would also yield results as in Fig. 1A (dashed lines) even without central auditory-system plasticity. More studies are needed to reveal the sources of the differences in the results.

While compensatory plasticity may or may not occur as illustrated in Fig. 1, there is no doubt that CHL exerts a persistent effect on the neural coding of spatial information in ICC neurons as demonstrated by the 37% reduction in the capacity of neurons to carry information about ILDs (Fig. 2). Reduced MI may suggest alterations in the responsiveness (spike rates), reliability (spike rate variability), as well as the general sensitivity of ICC neurons and/or their inputs to the cues to location, including ILD. The results suggest that at least for ICC neurons contralateral to the CHL a reduction in the capacity of ILD to modulate spiking was correlated with a reduction in information-carrying capacity of these neurons. The

impaired neural information processing demonstrated here may provide a basis for the persistent behavioural deficits in binaural and spatial hearing tasks that have been observed clinically after chronic CHL both during development and in adulthood. Since we have found persistent reductions in the ability of critical neural circuits in the ascending auditory pathway to encode spatial attributes of sound, it may logically follow that there will be a similar reduction in the perceptual capabilities as well. Towards this end, on-going studies are examining the behavioural consequences of reduced information processing due to CHL during development and in adults.

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