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Cochlear efferent feedback balances interaural sensitivity

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Neurons in the lateral superior olive (LSO) compute sound location based on differences in interaural intensity, coded in ascending signals from the two cochleas. Unilateral destruction of the neuronal feedback from the LSO to the cochlea, the lateral olivocochlear efferents, disrupted the normal interaural correlation in response amplitudes to sounds of equal intensity. Thus, lateral olivocochlear feedback maintains the binaural balance in neural excitability required for accurate localization of sounds in space.

The olivocochlear efferent pathway has two major subsystems (**Fig. 1a**,**b**): a medial (MOC) component of myelinated fibers projecting to the cochlea's outer hair cells and a lateral (LOC) component of unmyelinated fibers projecting to cochlear nerve fibers, near their afferent synapses with cochlear inner hair cells[1]. The MOC system is a cholinergic sound-evoked feedback loop, which, when activated, raises cochlear thresholds by decreasing the contributions of electromotile outer hair cells to the normal amplification of sound-induced vibration of the cochlear epithelium. The LOC system is cytochemically heterogeneous, with cholinergic, GABAergic, dopaminergic and peptidergic transmission. It comprises at least two subgroups: when activated, it elicits either slow ($\tau \sim 10$ min) excitation or slow suppression of cochlear nerve output[2].

To isolate LOC contributions to cochlear function, we stereotaxically lesioned the LSO unilaterally (right side) in mice (age 6–8 weeks) by injection of a neurotoxin (melittin[3]) and assessed effects bilaterally by physiological measures of cochlear neural excitability and outer hair cell function, 2 and 4 weeks later. The lesions were assessed double-blind in two ways (**Fig. 1**): (1) lesion location in serial brainstem sections stained for cholinergic markers and (2) density of olivocochlear efferent terminals in outer hair cell and inner hair cell areas in immunostained cochleas (**Supplementary Methods** online). Because LOC projections are almost exclusively to the ipsilateral inner hair cell area (**Fig. 1a**,**b**), when the injection successfully targeted the LSO (**Fig. 1d**), the cochlea on the injected side showed loss of cholinergic terminals in the inner hair cell area (**Fig. 1f**), without a change in cholinergic terminals on outer hair cells in either ear

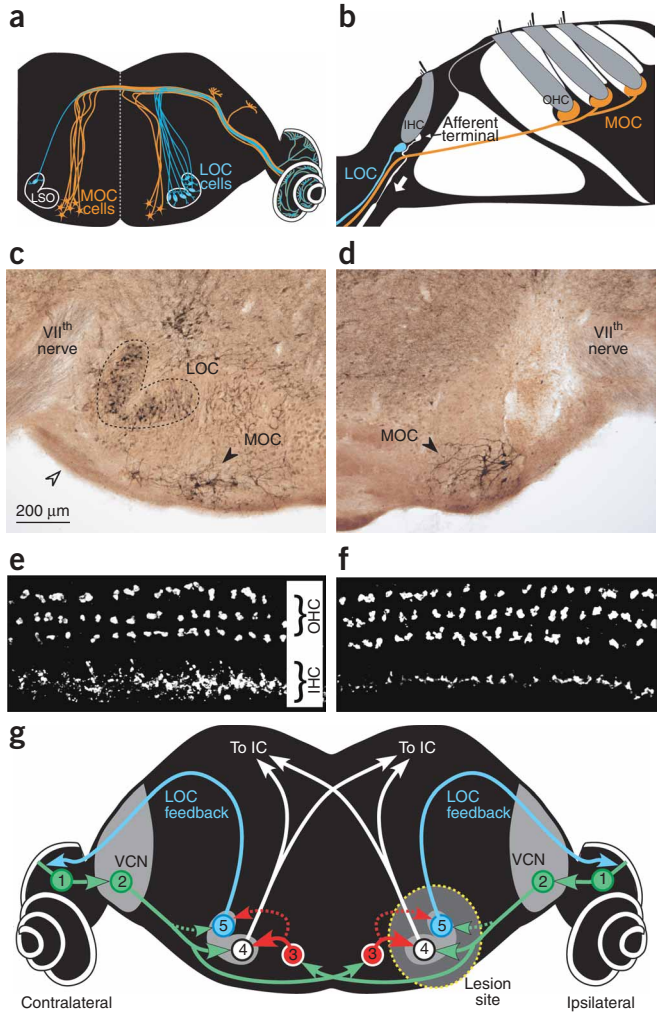

Figure 1 Assessment and interpretation of brainstem lesions. Schematics illustrate the central origins (**a**) and peripheral projections (**b**) of the medial and lateral components of the olivocochlear (OC) efferent system. Micrographs of an acetylcholinesterase-stained brainstem section ipsilateral (**d**) and contralateral (**c**) to a neurotoxin injection show the successful targeting of the LSO in one case and the corresponding selective loss of cholinergic immunostaining in the inner hair cell (IHC) area ipsilateral to the injection (**e** versus **f**). A schematic (**g**) illustrates the binaural circuitry driving the principal cells of the LSO: auditory nerve fibers (1) project to cochlear nucleus bushy cells (2), which send excitatory projections to the ipsilateral LSO (4) and inhibitory projections to the contralateral LSO via an interneuron (3) in the MNTB. Similar inputs to the nearby LOC somata (5) are hypothesized to account for the results in the present study. All procedures were approved by the Institutional Animal Care and Use Committee of the Massachusetts Eye and Ear Infirmary.

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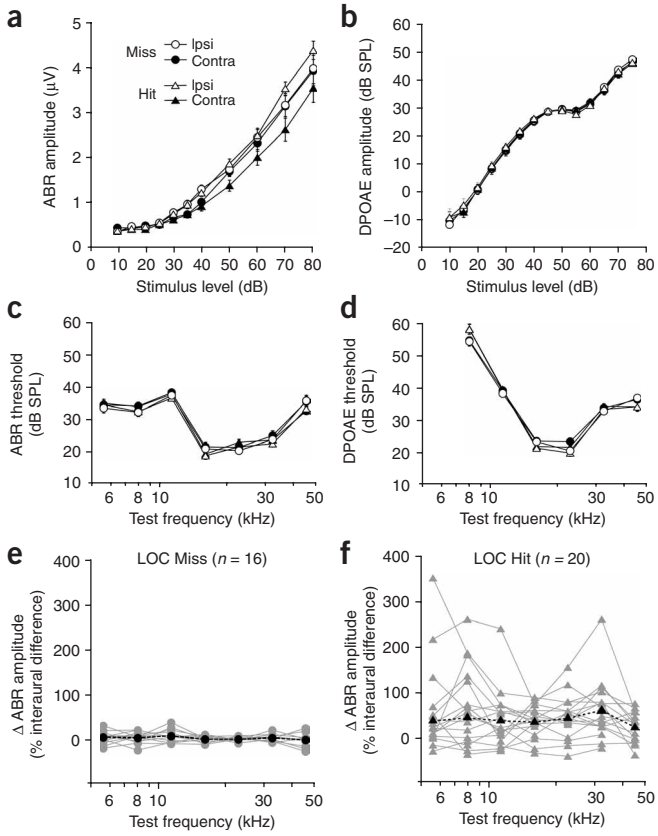

Figure 2 Unilateral cochlear de-efferentation disrupts interaural balance in neural excitability. Selective destruction of the LOC efferent system enhances mean cochlear neural response amplitudes (ABR) ipsilaterally and reduces them contralaterally (**a**) without affecting hair-cell based DPOAEs (**b**) or mean cochlear thresholds, as seen by either ABR (**c**) or DPOAE (**d**) measures. Comparing neural amplitudes between the two ears reveals that the normal binaural balance of excitability seen with an intact LOC (**e**) is disrupted when the LOC is destroyed (**f**). **a**–**d** show mean data (±s.e.m.) for different groups: symbol key in **a** applies to these 4 panels. Data in **a** and **b** are for stimuli at 22.6 kHz. **e**,**f** show data from individual cases (gray) and mean data for each group (black); each point is the mean interaural difference in ABR amplitude (expressed as the percent by which ipsilateral amplitudes exceed contralateral amplitudes) averaged, for one frequency, over the highest four levels presented (50–80 dB SPL). All ABR amplitudes reflect the peak-to-peak value of Wave 1, the summed activity of auditory nerve fibers.

(**Fig. 1e**,**f**). Based on this combined central and peripheral assessment, we concluded that, of the 36 mice that survived the initial surgery and all subsequent electrophysiological testing, 20 were at least partial 'hits' and 16 were complete 'misses'. In most miss cases, there was no lesion, suggesting that the injection pipet clogged. In one hit and one miss case, there was minor damage to the MOC system, as evidenced by proximity of the lesion to their cells of origin and by a just-detectable decrease in cholinergic terminals in the outer hair cell area.

To evaluate the effects of selective LOC de-efferentation, we took two measures of cochlear function[4]. The first, auditory brainstem response (ABR), constitutes the summed neural activity in the ascending auditory pathway in response to short tone bursts. The first deflections of this compound neural response (wave 1) represent the summed activity of the cochlear nerve, the sensory output of the inner ear[5]. The second measure, distortion product otoacoustic emissions (DPOAEs), is generated 'upstream' of the neural response and provides a sensitive measure of outer hair cell function. DPOAEs, as measured here, are distortion components generated in mechanoelectric transduction when two tones close in frequency are presented to the ear; these distortions are amplified by outer hair cell electromotility and transmitted back to the ear canal, where they can be measured in the sound pressure waveform.

As expected from previous work[2] and consistent with its peripheral projections, loss of the LOC system resulted in changes in auditory-nerve excitability (**Fig. 2a**) without alteration in outer hair cell function (**Fig. 2b**). Mean amplitude-versus-level functions for Wave 1 of the ABR (representing the summed activity of auditory nerve fibers) were significantly altered by the lesion (**Fig. 2a**); in hit cases, amplitudes ipsilateral to the lesion were significantly higher than those contralateral to the lesion ($P < 0.0001$ by two-way ANOVA), whereas interaural differences were not significant in the miss cases.

An unexpected result, given the overwhelmingly ipsilateral projection of the LOC system[6] (**Fig. 1a**), was that the changes in neural excitability were bilateral and complementary: contralateral to the lesion, amplitudes were slightly lower than control, whereas ipsilateral amplitudes were slightly enhanced. The study design included two types of controls: (1) right and left ears from age- and sex-matched animals without any surgical procedures, and (2) ipsilateral and contralateral ears from miss cases. Response amplitudes were indistinguishable in both types of controls; only the latter is shown in **Figure 2**.

Data in **Figure 2a** are for one test frequency (22.6 kHz): to evaluate interaural neural changes across frequency, we averaged each ipsi/contra pair of ABR amplitude functions across levels into a single value, the mean interaural amplitude ratio (**Supplementary Fig. 1** online), and plotted that value versus frequency. Viewed in this way (**Fig. 2e**,**f**), the data suggest that an intact LOC system balances neural excitability in the two ears. In normal ears, when response amplitudes are higher (or lower) than average in one ear (or in one frequency region of one ear), the opposite ear shows the same deviation, thus the ipsilateral/contralateral ratio remains close to unity (**Fig. 2e**). Loss of the LOC system (**Fig. 2f**) abolishes this tight interaural correlation in neural excitability seen in normal ears.

The complementary binaural effects seen after unilateral LOC ablations suggest, first, that LOC feedback can titrate excitability upward or downward and, second, that LOC circuitry must include contralateral inputs. A simple hypothesis for an LOC feedback circuit (**Fig. 1g**) can provide a framework to explain the results. Although inputs to LOC neurons are poorly characterized[7], their location within the LSO[8] might allow them to sample the same binaural inputs that drive nearby LSO principal cells[9]: LSO 'EI' cells compare excitatory (E) inputs from the ipsilateral cochlear nucleus (cell 2 in **Fig. 1g**) to inputs from the contralateral cochlear nucleus processed via an inhibitory interneuron in the medial nucleus of the trapezoid body (MNTB; cell 3). We hypothesize (**Fig. 1g**) that LOC cells (cell 5) process similar binaural EI inputs, but via a very slow (tens of minutes) integrator and that net LOC feedback is inhibitory. With such a circuit, interaural disparities are corrected: for example, a slow increase in auditory nerve excitability, including increased background rate, increases negative feedback ipsilaterally, and (via the sign change in the MNTB) decreases negative feedback contralaterally. Note that slow changes occurring downstream of the cochlear nerve, for example, in the cochlear nucleus, could also be corrected by this feedback circuit. To explain the complementary shifts in neural excitability in the two ears after unilateral LSO ablation, we note that a lesion that removes the entire LSO ipsilaterally could also interrupt the inhibitory input to the contralateral LOC (ipsilateral 2 to contralateral 3 in **Fig. 1g**), thereby reducing the resting level of inhibition ipsilaterally and increasing inhibition contralaterally.

Bilateral comparison by LSO cells mediates the computation of interaural level differences (ILDs), a major cue for localization of sounds in the azimuthal plane. ILDs arise because the head produces an asymmetrical attenuation of sounds off the center axis. Mice can resolve azimuthal differences of $\sim 15^{\circ}$ at 15 kHz (ref. 10), which corresponds to an ILD < 1 dB (ref. 11). For a typical mouse cochlear nerve fiber, a 1 dB level step changes discharge rate by less than 6% (ref. 12). Thus, the interaural excitability mismatches of 50 to 100% (and more) in de-efferented animals (**Fig. 2f**) should seriously degrade the accuracy of ILD computation. Our results provide the first evidence that the LOC system may provide a 'binaural balance' adjustment required for accurate sound localization[1]. Such a role would explain why so many studies of auditory processing have failed to reveal dramatic effects of cochlear de-efferentation[13]: few used tests that engage the binaural hearing system, and those that did, failed to sever the LOC system[14].

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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