Supplementary Materials and Methods

Animals and Groups Experimental animals were CBA/CaJ mice aged 6-8 weeks, weighing between 25-30 g, and of either sex. One group underwent surgery to unilaterally lesion the LSO: of these 36 survived. Histological evaluation revealed that in 16 cases there was no lesion site or the injection missed the LSO; in 20 cases the lesion caused at least partial loss of the LOC system. An additional 4 age- and sex-matched ears were used as non-surgical controls: their housing conditions and test schedules were otherwise identical. All procedures were approved by the IACUC of the Massachusetts Eye and Ear Infirmary.

Surgery: Following anesthesia (xylazine 20 mg/kg and ketamine 100 mg/kg i.p.) the mouse was held in a small-animal stereotaxic apparatus, the skin overlaying the skull was slit to reveal bregma and lambdoidal sutures, which are positioned in the horizontal plane. A hole in the skull was positioned 0.49 \text{ mm} \text{ caudal and 0.12 mm} \text{ lateral to the bregma. A micropipette filled with 10\% mellitin in saline was advanced in the vertical plane to a depth of 0.69 mm, and 2 \text{ l} \text{ was injected via a syringe coupled to the back of the micropipette. Immediately after injection, the scalp was sutured, and the animal placed in a padded cage for recovery.}

ABR and DPOAE assays: At 2 and 4 wks after surgery, animals were re-anesthetized for bilateral measurement of auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). Ears were tested sequentially: stimuli were always monaural. For ABRs, needle electrodes were inserted at vertex and pinna and 5-ms tone pips (0.5-ms rise–fall, \text{cos}^2 \text{ envelope}) were presented at 35/sec. The response was amplified (10,000x), filtered (0.1–3 kHz), and averaged with an digital I-O board in a PC-based data-acquisition system. Sound level was raised in 5-dB steps from 0 to 80 dB SPL. At each level, 1,024 responses were averaged (with stimulus polarity alternated) after ‘artifact rejection’. Threshold was determined by visual inspection. The DPOAE at 2f_1-f_2 was recorded in response to two primary tones: f_1 and f_2, with f_2=f_1 * 1.2 and with f_2 \text{ level} = f_1 \text{ level} -10 \text{ dB SPL}. Ear-canal sound pressure was amplified and digitally sampled by the same I-O board producing the primary tones. Fast-Fourier transforms were computed from averaged waveforms of ear-canal sound pressure, and the DPOAE amplitude at 2f_1-f_2 and surrounding noise floor were extracted. Iso-response contours were interpolated from plots of amplitude vs. sound level, performed in 5-dB steps of f_1 level. Threshold is defined as the f_1 level required to produce a DPOAE of 5 dB SPL.

Histological assessment of lesions: After final testing, all animals were perfused with 10\% formalin. Brainstems were extracted, post-fixed, cryoprotected, frozen and cut on a sliding microtome at 80 \text{ m} \text{ in the transverse plane. Slide-mounted sections were stained for acetylcholinesterase (AChE) activity to allow for visualization of the cholinergic LOC cells in the LSO, and to verify that the injection pipette did not sever the OC bundle. The success of the LSO lesion was quantified by tracing the outline of surviving LSO cells in all relevant sections on both injected and control sides. Tracings were digitized, and the areas of medial and lateral limbs determined by computerized planimetry. Cochleas were extracted, post-fixed overnight, decalcified in EDTA for \sim48 \text{ hrs and then dissected into half-turn segments. Segments were incubated in primary antibody overnight (rabbit anti-VAT: vesicular acetylcholine transporter) followed by a fluorescently labeled secondary antibody (Alexafluor 568). Fractional survival of VAT-positive terminals was then analyzed semi-quantitatively by an observer blinded to case history.}