Supporting Information

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SI Methods

Animals. Wistar rats aged between 15 and 22 d old were used. Experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act of 1986 Home Office regulations (1).

Acoustic Overexposure. Rats were anesthetized with an i.p. injection of fentanyl (0.15 mg/kg; VetaPharma Ltd), fluanisone (5 mg/kg; VetaPharma Ltd.), and Hypnovel (2.5 mg/kg; Roche) and placed in a custom-made open-field sound-insulated chamber containing a 600-W High Power Horn Tweeter (frequency range: 2–20 kHz; Maplin) and delivering a pure tone (14.8 kHz) at 110 dB sound pressure level (SPL) for 2 h. Two sessions of 2 h of acoustic overexposure (AOE) were performed at P15–P18, which corresponds to the period after the hearing onset (2). A 1-d interval was left between the two sessions. Control animals were similarly anesthetized but unexposed to AOE.

Auditory Brainstem Response. Auditory brainstem responses (ABR) were evoked on anesthetized rats by calibrated tone pips (8/12/16/24/30 kHz; 1-ms rise and fall times, 5-ms duration, 3-ms plateau) generated in free field at 10 Hz by a waveform generator (TGA 1230 30 MHz; Tucker Davis Technology) and an acoustic driver (Bruel & Kjaer type 4192). The acoustic driver was placed at a 1-cm distance from the rat's ear. Evoked responses were recorded by an amplifier (Medelec Sapphire 2A; Oxford Instruments), filtered between 10 Hz and 5 kHz and averaged with 300–400 sweeps using a custom-made software (GlaxoSmithKline). Tone pips were progressively attenuated in 10–3 dB SPL steps from an initial intensity of 94 dB SPL using a digital attenuator (PA4; Tucker Davis Technology). The hearing threshold was defined as the lowest intensity yielding the consistent appearance of ABR peak I and II. The threshold shift was measured as a difference between the hearing threshold measured on day 1 (P15–P18) and the hearing threshold measured 3 or 4 d later.

Whole-Cell Recordings. Coronal brainstem slices (170 μm) containing the dorsal cochlear nucleus (DCN) were obtained from Wistar rats (P18–P22) in low Na^+ artificial cerebrospinal fluid (ACSF) with 0.1. mM Ca^{2+} and 4 mM Mg^{2+} as previously described (3). Whole-cell current clamp recordings were performed from fusiform cells (FCs), and granule cells were identified on the basis of morphological and electrophysiological properties (4, 5) using a Multiclamp 700A amplifier (Molecular Devices Inc.) with a sampling rate of 20 kHz, filtering at 5 kHz and using PClamp 9 software (Molecular Devices Inc.). Recordings were carried out in normal ACSF (3) with 2 mM Ca^{2+} and 1 mM Mg^{2+}. Inhibitory inputs were blocked with 10 μM strychnine and 20 μM gabazine, and excitatory inputs were blocked with 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 50 μM D-AP5. The amplitude of the excitatory post-synaptic potentials (EPSPs) was measured in the presence of strychnine and bicuculline whereas the amplitude of the inhibitory post-synaptic potentials (IPSPs) was obtained by subtracting the EPSP in isolation (recorded in strychnine and gabazine) from the post-synaptic potentials recorded in control medium. The pipette (4–6 ΩM) contained (in mM): K-gluconate 130; KCl 0.5; EGTA 5.4; Hepes 10; $MgCl_2$ 1; NaCl 2; 0.1% Lucifer yellow (adjusted to pH of 7.1–7.3 with KOH). Signals were corrected off-line for the liquid junction potential (−11 mV). All recordings were performed at 25 °C. Auditory nerve (AN) and multisensory (MS) fiber tracts (Fig. S5) were stimulated using a voltage stimulator (DS2A; Digitimer) delivering 100-μs, 5- to 50-V pulses at a rate of 0.3–100 Hz to a bipolar electrode (FHC Inc.) placed on the synaptic pathways (4). The post-synaptic potential threshold in FCs was defined as the minimal stimulus voltage necessary to elicit a depolarization. The stimulus strength was then increased by 5 and 10 V to recruit additional inputs to the FC. When stimulating at 10–100 Hz, the action potential threshold was defined as the minimal voltage to elicit few (1 to 7) action potentials in FCs (threshold). Stimulations were further increased by 5–10 V to allow for fiber recruitment (suprathreshold). Gabazine was from Tocris; all of the other chemicals were from Sigma.

Spike Analysis. Firing rates after step current injections were fitted with the sigmoidal function $y = a/(1 + \exp(-(x - x0)/b))$, where x is the current (in pA), $x0$ is the point of inflection of the curve, y is the frequency (in Hz), a is the maximal frequency, and b is the slope (firing gain). Firing rates after synaptic stimulations (input–output relationships) were fitted by the Hill equation $y = F_{max}/(1 + 10((\text{Log } X_{50} - X)^*n))$, where y is the response (Hz), F_{max} is the maximum firing rate, X is the logarithm of the input frequency (Hz), X_{50} is the value of X at which F reaches half maximum, and n is the Hill coefficient (slope).

Neuronal Model. A Leaky Integrate and Fire model was implemented in MATLAB (Mathworks) to simulate the firing of FCs evoked while stimulating the multisensory and auditory fibers. V reset and V threshold (V th) were set up at 0 and 20 mV, respectively, and the refractory period was fixed at 2.5 ms. See Tables S5 and S7 for a detailed description of all of the parameters used in the model.

Transmitted Electron Microscopy. Wistar rat cochlea's were removed and fixed (>24 h) in 2.5% glutaradehyde and 2% paraformaldehyde in 0.2 M Sörensens buffer at (pH 7.4). Samples were decalcified in 8% EDTA. Following decalcification, samples were rinsed, postfixed in osmium tetroxide (1%) in 0.05 M buffer, and embedded in epoxy resin. Embedded samples were sectioned transversely using an Ultracut S ultramicrotome (Reichert). Thin sections of ~80-nm thickness were cut and collected onto copper mesh grids. Each grid was counterstained with 2% uranyl acetate and Reynold's lead. Samples were viewed on the JEOL 1220 transmitted electron microscopy with an accelerating voltage of 80 kV. Images were captured using Magaview III digital camera (Olympus Soft Imaging Solutions). Rynold's lead was made in house (6). Propylene oxide and lead citrate components were from Fisher Scientific. All other chemicals were from Agar Scientific.

Tridimensional Reconstruction of the Cochlear Nucleus. A tridimensional reconstruction of the whole cochlear nucleus was obtained from staining thin (20-μm) slices with cresyl violet. After decapitation, the brainstem and cerebellum were separated from the whole brain (as described above), transferred in Tissue-tek (polyvinyl alcohol <11%, carbowax <5%, nonreactive ingredients >85%), and fast-frozen in dry ice (in hexane). Tissue was then left for at least 1 d at −21 °C. The frozen brainstem was cut into 20-μm-thick sections using a freezing microtome. Sagittal slices were collected and transferred onto polysine slides. Slides containing the cochlear nucleus slices were then transferred into a solution containing 5% paraformaldehyde dissolved into phosphate buffer (PBS, pH 7.4) for 15 min and maintained under agitation. The slides were washed in 0.1% PBS (pH 7.4) and put for another 15 min in 5% paraformaldehyde. Slides were then stained for 4–5 min with 0.1% cresyl violet (dissolved in distilled water) and maintained under agitation. The slides were then immediately transferred into pure ethanol for 2 min and into

xylene for another 2 min before being mounted in DPX (Dibutyl phthalate-Polystyrene-Xylenes) and covered with a coverslip.

Image Acquisition and Processing. All of the images used for this study were acquired with a Nikon DXM1200F digital camera on a Nikon Eclipse TE2000-U inverted microscope. To allow the reconstruction of the cochlear nucleus, 50 consecutive images were perfectly overlapped on a monitor and acquired with Reconstruct software (ImageJ). Areas were outlined with a freehand selection tool (ImageJ version 1.36) to select the DCN, the ventral cochlear nucleus (VCN), the auditory nerve, and granule cell domains.

Movie. Movie S1 summarizes the effect of stimulating by the auditory or multisensory inputs on the firing of a fusiform cell. The audio was constructed using a granulation technique (7) to sonify the firing events of the FC in the Leaky Integrate and Fire model simulations. Each time the cell fires, a small grain of sound of duration 50–200 ms is sounded (8).

1. Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. UK Home Office.
2. Geal-Dor M, Freeman S, Li G, Sohmer H (1993) Development of hearing in neonatal rats: Air and bone conducted ABR thresholds. *Hear Res* 69:236–242.
3. Barnes-Davies M, Barker MC, Osmani F, Forsythe ID (2004) Kv1 currents mediate a gradient of principal neuron excitability across the tonotopic axis in the rat lateral superior olive. *Eur J Neurosci* 19:325–333.
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5. Pilati N, Barker M, Panteleimonitis S, Donga R, Hamann M (2008) A rapid method combining Golgi and Nissl staining to study neuronal morphology and cytoarchitecture. *J Histochem Cytochem* 56:539–550.
6. Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol* 17:208–212.
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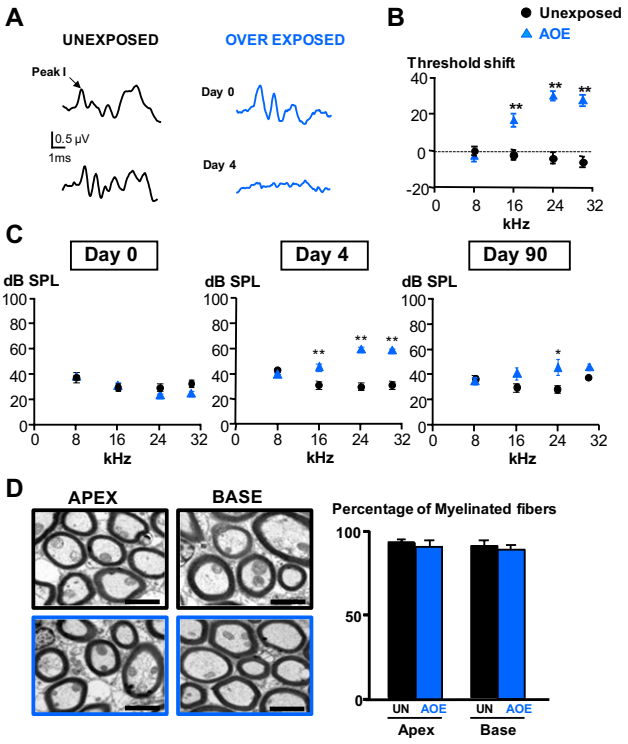

Fig. S1. (*A*) Examples of ABR elicited by a 24-kHz tone tip of 64 dB SPL measured before (*Upper*, day 0) and 4 d after exposure or anesthesia (*Lower*, day 4). (*B*) Summary of ABR thresholds shifts for 8- to 30-kHz frequencies from 13 unexposed and 20 exposed rats. (*C*) Summary of ABR threshold (8–30 kHz) measured at day 0, day 4, and day 90 (after the exposure to loud sound) in unexposed and over exposed rats. (*D*) (*Left*) Transmitted electron microscopy photographs of peripheral auditory nerve fibers in unexposed condition (black frame) and 90 d after AOE (blue frame) at the base and at the apex of the cochlea. (*Right*) Histograms represent the percentage of myelinated AN fibers in those conditions. (Scale bar: 1 µm.) $*P < 0.05$,$**P < 0.01$: unpaired *t* test.

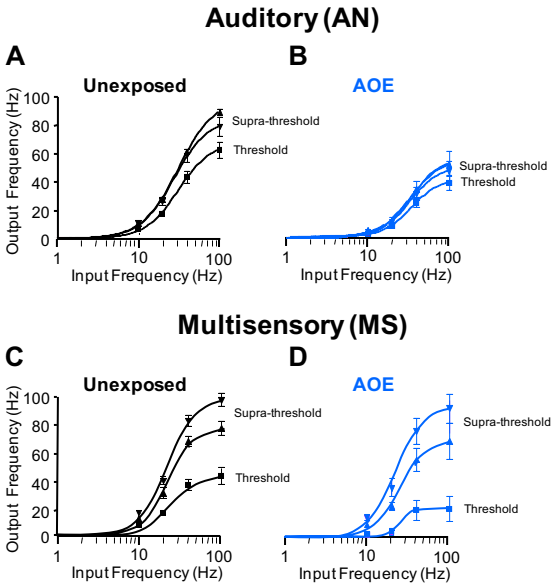

Fig. S2. FC input–output relationship after stimulating auditory (AN) and multisensory (MS) inputs with increasing stimulating voltages. AN inputs (*A* and *B*) and MS inputs (*C* and *D*) were stimulated from 10 to 100 Hz. Average input–output relationships (n = 5–6) were obtained in unexposed condition (black) and after AOE (blue) in strychnine and gabazine. Lines are fits to a Hill function as detailed in *SI Methods*. Stimulating voltages were the following: AN (40, 45, and 50 V) and MS (20, 25, and 30 V).

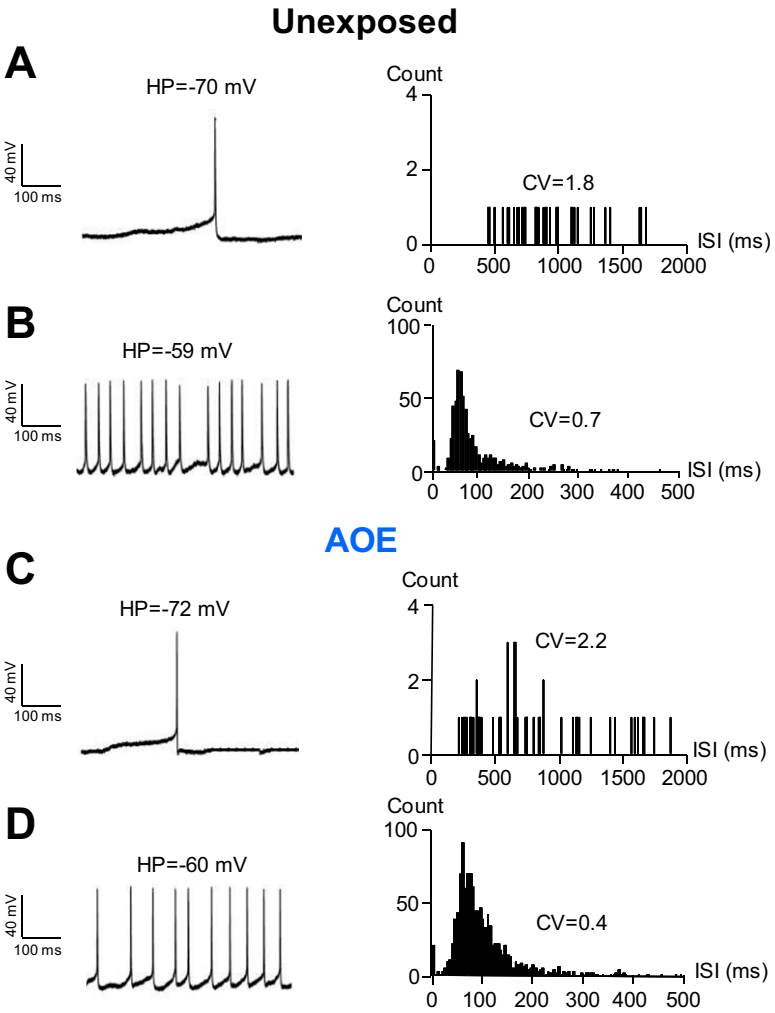

Fig. S3. Firing properties of two granule cells originating from Wistar rats unexposed (*A* and *B*) or previously exposed to a loud single tone (*C* and *D*). Action potentials were taken at threshold (*A* and *C*) and at maximal frequency (*B* and *D*). (*Left*) Examples of granule cell firing with cells firing at 0.7, 11, 0.4, and 10 Hz for *A*, *B*, *C*, and *D*, respectively. Membrane potentials are indicated above the traces. (*Right*) Histograms showing the distribution of the interspike intervals (ISI) for those cells and the coefficient of variation (CV). Note the absence of the effect of acoustic overexposure on the firing of granule cells (Table S8).

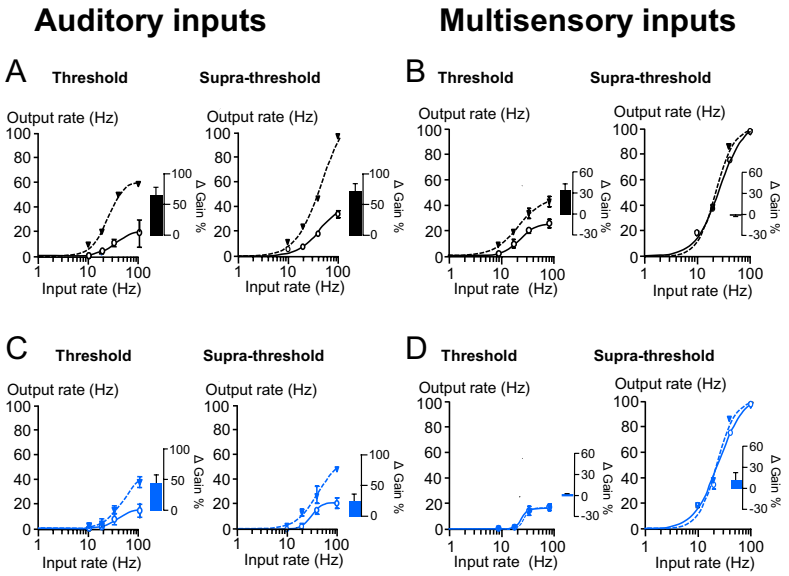

Fig. S4. Computational modeling reproducing the effect of AOE on the gain control after removal of inhibitory synaptic transmission. Modeled input–output relationships are represented in unexposed (black) and after AOE (blue), and lines are fits to a Hill function as detailed in *SI Methods*. Input–output relationships are represented following AN stimulations (*A* and *C*) and MS stimulations (*B* and *D*) stimulations before (solid line) and after (dashed line) removal of synaptic inhibition. Histograms represent the changes in the gain before and after removal of synaptic inhibition.

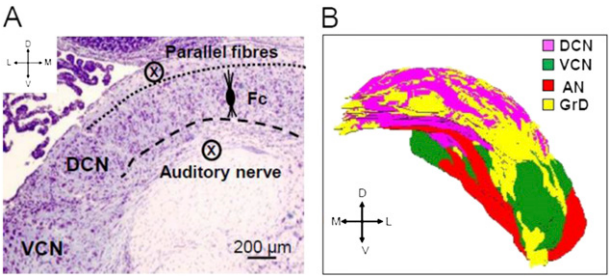

Fig. S5. (*A*) Coronal slice of the cochlear nucleus labeled with cresyl violet (5). A circled "x" indicates the location of the stimulation electrode positioned either on the parallel fibers (multisensory inputs) in the molecular layer or on the auditory nerve fibers in the deep layer. The dotted and dashed lines are approximate boundaries between the molecular layer and the fusiform cell layer and between the deep layer and the fusiform layer, respectively. (*B*) Three-dimensional reconstruction of the cochlear nucleus. Coronal serial reconstruction of the cochlear nucleus. The DCN is in pink and the VCN is in green; the granule domains (GrD) are labeled in yellow, and the AN is labeled in red.

Table S1. Analysis of the ABR wave I and II latency elicited by single-tone pip (24 and 8 kHz) delivered at 74 dB SPL in unexposed rats

	Unexposed day 0 (n = 8)		Unexposed day 4 (n = 8)	
Frequency	Latency wave I (ms)	Latency wave II (ms)	Latency wave I (ms)	Latency wave II (ms)
24 kHz	1.5 ± 0.21	2.4 ± 0.30	1.4 ± 0.04 (NS)	2.3 ± 0.06 (NS)
8 kHz	1.7 ± 0.07	2.8 ± 0.08	1.6 ± 0.07 (NS)	2.5 ± 0.11 (NS)

ABR were measured at day 0 and at day 4. Paired Student's t test was performed. Values are represented as mean ± SEM, and n refers to the number of rats used for this analysis. NS: not significant ($P > 0.05$).

Table S2. Analysis of the ABR wave I and II latency elicited by single-tone pip (24 and 8 kHz) delivered at 74 dB SPL in exposed (AOE) rats

	AOE day 0 (n = 7–8)		AOE day 4 (n = 7–8)	
Frequency	Latency wave I (ms)	Latency wave II (ms)	Latency wave I (ms)	Latency wave II (ms)
24 kHz	1.5 ± 0.05	2.5 ± 0.08	1.5 ± 0.09 (NS)	2.3 ± 0.09 (NS)
8 kHz	1.8 ± 0.07	2.5 ± 0.13	1.6 ± 0.05 (NS)	2.4 ± 0.07 (NS)

ABR were measured at day 0 and at day 4. Paired Student's t test was performed. Values are represented as mean ± SEM, and n refers to the number of rats used for this analysis. NS: not significant ($P > 0.05$).

Table S3. Analysis of the ABR wave I and II latency elicited by single-tone pip (24 and 8 kHz) delivered at 74 dB SPL in unexposed rats (left) and exposed rats (right)

	Unexposed day 4 (n = 8)		AOE day 4 (n = 7–8)	
Frequency	Latency wave I (ms)	Latency wave II (ms)	Latency wave I (ms)	Latency wave II (ms)
24 kHz	1.5 ± 0.05	2.5 ± 0.08	1.5 ± 0.09 (NS)	2.3 ± 0.09 (NS)
8 kHz	1.8 ± 0.07	2.5 ± 0.13	1.6 ± 0.05 (NS)	2.4 ± 0.07 (NS)

Same values as in Tables S1 and S2 (day 4) are reported to allow comparison between unexposed and AOE at day 4. Unpaired t test was performed. Values are represented as mean ± SEM, and n refers to the number of rats used for this analysis. NS: not significant ($P > 0.05$).

Table S4. Analysis of FC action potential thresholds (elicited by step current injections) and of EPSP amplitudes (elicited by AN stimulations)

	FC unexposed (n = 4–8)	FC AOE (n = 5–8)
Step current injections		
Action potential threshold (mV)	−44.5 ± 1.1	−42.5 ± 1 (NS)
Action potential threshold (pA)	200 ± 23	212 ± 21 (NS)
Synaptic stimulations		
EPSP amplitude (mV)	10.6 ± 1.4	3.9 ± 0.7 ($P < 0.01$)
Stimulating voltage (V)	42 ± 5	56 ± 4 ($P < 0.05$)

Action potential threshold was measured at the threshold membrane potential (mV) and also at the minimal step current (in pA), triggering an action potential. EPSP amplitude (mV) was measured at the highest stimulating voltage (V), evoking the EPSP without triggering an AP. Note that, after AOE, action potential thresholds were left unchanged whereas EPSP amplitudes were smaller (even at higher stimulating voltages). Values are represented as mean ± SEM, and unpaired Student's t tests were performed.

Table S5. Parameters used in the Leaky Integrate and Fire model

	Auditory fibre stimulations					
	AN	Tv	AN-Tv	AN-Fu	Tv-Fu	AN
	Cell number		*Synaptic strength*			*(GΩ)*
UN	100	20	40	40	40	1
AE	53	20	5	40	4	1

The model comprises three passive property parameters (R_m, the membrane resistance; C_m, the membrane capacitance; and τ_m, the membrane time constant resulting from $C_m.R_m$). Passive properties of fusiform cells (FC), auditory nerve (AN), and tuberculo ventral cells (Tv) were determined experimentally as follows: (FC: R_m = 50 MΩ and C_m = 200 pF, giving a τ_m of 10 ms; AN R_m = 1,900 MΩ, C_m = 10 pF, giving a τ_m of 19 ms; Tv: R_m = 100 MΩ, C_m = 100 pF, giving a τ_m of 10 ms). Auditory nerve fibers were given the theoretical values of R_m = 1,000 MΩ and C_m = 10 pF, giving a τ_m of 10 ms. All these parameters were fixed during the simulations. The number of AN fibers projecting onto a single FC was determined according to previous anatomical studies reporting that one FC receives synaptic projections from 10 AN fibers (and 10 terminals) (1). Spatial fiber recruitment was simulated by varying the inactivation probability (from 0 to 0.49) so that the zero value corresponds to the totality of cells being recruited. The pharmacological action of strychnine and gabazine on the inhibitory synaptic transmission was simulated by applying a factor 0 to the strength of the inhibitory synapses. Blue circles indicate the parameters that were altered to reproduce the effects of AOE on the FC transfer function after AOE.

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Table S6. Percentage of myelinated AN fibers at the base and the apex of the cochlea in unexposed condition and 3 and 90 d after AOE

	Day 3		Day 90	
	Unexposed (%)	AOE (%)	Unexposed (%)	AOE (%)
Apex	88.8 ± 2.5	90.9 ± 1.8 (NS)	91.9 ± 2.7 (NS, NS)	91.0 ± 3.7 (NS, NS, NS)
Base	89.6 ± 3.0	50.9 ± 5.8 ($P < 0.001$)	90.7 ± 4.1 (NS, $P < 0.001$)	88.1 ± 3.5 (NS, $P < 0.001$, NS)

"Day 3 unexposed" refers to 3 d after initial ABR recording, and "day 3 AOE," which refers to 3 d after initial ABR recording and AOE. "Day 90 unexposed" refers to 90 d after initial ABR recording, and "day 90 AOE" refers to 90 d after acoustic exposure. Values are reported ± SEM. "Day 3 AOE": value in parentheses refers to comparison with "day 3 unexposed." "Day 90 unexposed": first value in parentheses refers to comparison with "day 3 unexposed"; second value in parentheses refers to comparison with "day 3 AOE." "Day 90 AOE": first value in parentheses refers to comparison with "day 3 unexposed"; second value in parentheses refers to comparison with "day 3 AOE"; and third value in parentheses refers to comparison with "day 90 control". *P* values were obtained with one-way ANOVA (Tukey post hoc) tests. NS, nonsignificant for *P* values >0.05.

Table S7. Parameters used in the leaky integrate and fire model

	Parallel fibre (multisensory) stimulations					
	gr	Cw	gr-Cw	gr-Fu	Cw-Fu	gr
	Cell number		*Synaptic strength*			*(GΩ)*
UN	5000	20	25	25	25	1.9
AE	5000	20	25	25	0.3	1.1

The model comprises three passive property parameters (R_m, the membrane resistance; C_m, the membrane capacitance; and τ_m, the membrane time constant resulting from $C_m.R_m$). Passive properties of fusiform cells (FC), granule cells (gc), and cartwheel cells (Cw) were determined experimentally as follows: (FC: R_m = 50 MΩ and C_m = 200 pF, giving a τ_m of 10 ms; gc: R_m = 1,900 MΩ, C_m = 10 pF, giving a τ_m of 19 ms; Cw: R_m = 100 MΩ, C_m = 100 pF, giving a τ_m of 10 ms). R_m for gc was decreased to 1,100 MΩ after AOE. The number of gc's projecting onto a single FC was determined according to previous anatomical studies reporting that one FC receives synaptic projections from 10,000 parallel fibers (1–3). Spatial fiber recruitment was simulated by varying the inactivation probability (from 0 to 0.49) so that the zero value corresponds to the totality of cells being recruited. The pharmacological action of strychnine and gabazine on the inhibitory synaptic transmission was simulated by applying a factor 0 to the strength of the inhibitory synapses. For the sake of simplicity, the model will refer to gc as the cell bodies of the parallel fibers. Blue circles indicate the parameters that were altered to reproduce the effects of AOE on the FC transfer function after AOE.

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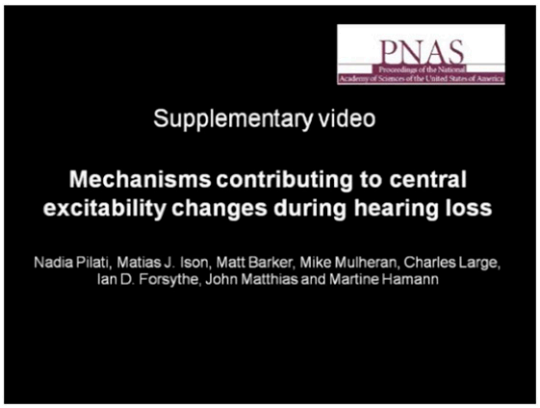
2. Napper RM, Harvey RJ (1988) Number of parallel fiber synapses on an individual Purkinje cell in the cerebellum of the rat. *J Comp Neurol* 274(2):168–177.
3. Harvey RJ, Napper RMA (1991) Quantitative studies on the mammalian cerebellum. *Prog Neurobiol* 36:437–463.

Table S8. Analysis of the spontaneous action potential firing of granule cells

	Granule cells unexposed (n = 7)	Granule cells AOE (n = 8)
Firing frequency (Hz) at threshold	0.8 ± 0.3	0.9 ± 0.4 (NS)
CV at threshold	1.9 ± 0.4	2.2 ± 0.5 (NS)
V_m (mV) at threshold	−71 ± 5.1	−73 ± 2.9 (NS)
Firing frequency (Hz) at F_{max}	9.1 ± 1.5	14 ± 2.9 (NS)
CV at F_{max}	0.7 ± 0.1	0.4 ± 0.1 (NS)
V_m (mV) at F_{max}	−63.0 ± 3.4	−60.0 ± 1.7 (NS)
Amplitude (mV)	70 ± 4.3	74 ± 2.6 (NS)
10–90% rise time (ms)	15.7 ± 5.9	10.3 ± 3.3 (NS)
90–10% decay time (ms)	1.95 ± 0.15	2.4 ± 0.5 (NS)

Action potential firing properties were quantified by the frequency, the coefficient of variation (CV), and the membrane potential (*V*m) measured at action potential threshold and maximal frequency (F_{max}). Action potential characteristics were described by the amplitude, 10–90% rise time, and 90–10% decay time. Values are represented as mean ± SEM, and unpaired Student's *t* test was performed. NS: not significant ($P > 0.05$).



Movie S1. Effect of acoustic over exposure on the firing of a fusiform cell. Fusiform cell firing is elicited by stimulating the multisensory inouts (part 1) and stimulating the auditory nerve (part 2).

Movie S1