

Supplementary Materials for

Multichannel optogenetic stimulation of the auditory pathway using microfabricated LED cochlear implants in rodents

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Other Supplementary Material for this manuscript includes the following:

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Movie S2 (.mp4 format). Visualization of oCI stimulation in the explanted rat cochlea.

Movie S3 (.mp4 format). Video of parts of a shuttle box session in a deaf, AAV-injected rat with the complete oCI system.

Materials and methods

Assembly and packaging.

oCI substrates (see fig. S2Ai) peeled from the handle wafer were individually turned into functional oCIs starting with the LED chip assembly (fig. S2Aii). Ten LED chips (Cree C460TR2227-S2100, Three Five (III-V) Materials Inc.) were sequentially flip-chip bonded with their n- and p-contacts on the corresponding pads on the PI substrate. The flip-chip bonder (Fineplacer 96λ, Finetech GmbH) offers an accuracy of $\pm 5\text{ }\mu\text{m}$; we applied a bonding force of 1.5 N at a temperature of 120°C and an ultrasonic energy of 300 mW. These process parameters ensure the mechanical and electrical interconnection of the LED bond pads to the 5- μm -high substrate pads. After LED assembly, we rinsed the oCIs with DI water before an Ar plasma (50 W, 1 min) cleaned and activated the PI surface (fig. S2Aiii). Immediately thereafter, the LEDs were underfilled with a solvent free epoxy (EPO-TEK 301-2, John P. Kummer GmbH) (fig. S2Aiv). The underfill was cured for 12 hours in an oven at 120°C. A first polymeric encapsulation using Cytop 809 M (Asahi Glass Co. Ltd.) was applied immediately after a further plasma treatment (Ar, 50 W, 1 min), as shown in figs. S2A, v and vi. A soft bake of this passivation was performed in an oven at 50°C for 30 min and 80°C for 1 h. This stage of fabrication including an additional hard bake step at 180°C for 1 h was used for the longevity testing (Fig. 2G). For the oCI samples used in vivo, an oxygen plasma (50 W, 1 min) was then applied (fig. S2Avii) to improve the layer adhesion of the final silicone (ELASTOSIL RT 604, Wacker Chemie AG and MED-6215, NuSil Technology LLC) passivation layers (fig. S2Aviii). The final annealing process was performed in two steps at 80 and 180°C, both applied for 1 h each.

The top view and cross-sectional schematics in fig. S2B illustrate the layered structure of the oCI and the used materials in the LED area. Figures S2, C and D, demonstrate the benefit of using a solvent-free underfill: when Cytop was used as underfill voids were observed underneath the LEDs caused by solvent evaporation and related material shrinkage, while the use of the solvent-free EPO-TEK 301-2 prevented void formation (fig. S2D).

Experimental characterization.

The optoelectronic characterization of the oCIs was performed with a custom-made setup consisting mainly of a current source and an optical measurement system. The current source was built using a diode driver (iC-HKB, iC-Haus GmbH) combined with an evaluation board from iC-Haus GmbH. The DAQ PCIe-6259 board (National Instruments) was controlled by a LabView program. The LED current supplied by the driver and the voltage-drop over the oCI were measured and stored via the LabView program. In parallel, the program controls the optical measurement system consisting of an integrating sphere (ISP-50-I-USB, Ocean Optics) combined with a spectrometer (USB4000, Ocean Optics). It enables the radiant flux Φ as well as the spectral distribution of the emitted light to be determined. For this purpose, the LED-carrying tip of the oCI was inserted into the 8-mm-wide aperture of the integrating sphere while varying the LED current I_{LED} from 0 to 45 mA at a DC of 10% and a frequency of 10 kHz for the time-averaged radiant flux measurements.

The thermal characterization was performed using the integrated Pt meander as a temperature sensing element. In a first calibration measurement, we extracted the resistance change of the Pt meander as a function of temperature between 22.5°C and 58.5°C. The resistance change was measured using a four-wire setup by applying a constant Pt meander current of 100 μA and

measuring the voltage-drop along the Pt meander. The extracted temperature coefficient of resistance was $2.71 \times 10^{-3} \text{ K}^{-1}$ at 37°C . In order to determine the temperature increase of an LED in operation, the oCI was sandwiched between two agarose gel slides kept at 37°C and simulating the thermal coupling of the probe to tissue. Measurements of the meander resistance and thus temperature as a function of time were performed while applying LED current pulses of various magnitudes I_{LED} at 100% DC and durations t_{on} . For each combination of I_{LED} and t_{on} , 16 measurements were averaged. By fitting exponential relaxation functions to the data, a thermal time constant of 11.8 ms was extracted. As a second relevant parameter, the asymptotic temperature increase of the Pt meander under the LED was determined as well. The thermal model of the oCI allows predicting the maximal temperature increase for a given LED current, pulse duration and stimulation frequency.

Passivation validation.

The characterization of the oCI encapsulation was performed with an automated leakage current test setup. Here, the oCI was connected via a ZIF connector to a printed circuit board (PCB) integrated into a tailored silicone plug of a test tube. The oCI was inserted into the test tube containing Ringer's solution simulating the environment of the probe in the cochlea. The leakage test was performed by applying the maximum recommended LED reverse bias voltage of -5 V while measuring the leakage current I_{leak} between the n- and p-lines of the LED using a source meter (Model 2602A, Keithley Instruments). The source meter was controlled via a GPIB interface using a LabView program storing the current value at predefined time intervals. The measurement was terminated once I_{leak} exceeded the set limit of $1.0 \mu\text{A}$ for 10 consecutive measurement points of I_{leak} .

Thermal simulation.

The simulation of the temperature distribution inside and outside the oCI is based on a finite element analysis (FEA) using SolidWorks Simulation Premium 2016. The model consists of the LED chip (SiC substrate and gold contact pads) and the PI substrate with bond pads and wiring, and a passivation based on epoxide (EPO-TEK) and silicone (fig. S3A). The oCI model is centered within a cube (edge length 1 cm; not shown in fig. S3A) filled with water mimicking the thermal coupling to the surrounding perilymph fluid inside the scala tympani. The lateral dimensions of the LED-based oCI model corresponds to those of the fabricated devices (fig. S3B). Component geometries were however simplified for meshing reasons. Material parameters are listed in table S1. It is assumed that temperature is continuous at all interfaces between materials. As boundary condition, we defined the surface temperature of the water cube to be constant with $T_{\text{surface}} = 37^\circ\text{C}$. Based on our electrical LED characterization, a heating power of $P_{\text{heat}} = 119.8 \text{ mW}$ is dissipated at the bottom surface of the LED with P_{heat} given by $P_{\text{heat}} = P_{\text{LEDelec}} - P_{\text{opt}}$, where $P_{\text{opt}} = 18.1 \text{ mW}$ denotes the emitted optical power and $P_{\text{LEDelec}} = 137.9 \text{ mW}$ the applied electrical power including resistive losses along the LED wiring. The chosen power corresponds to an LED current of $I_{\text{LED}} = 38.9 \text{ mA}$. The false color image in fig. S3A shows the simulated temperature distribution on the surface of the individual oCI components while fig. S3B illustrates the thermal image as a cross section through the oCI. The high thermal conductivity of the LED chip (SiC substrate) efficiently homogenizes the thermal profile across the LED chip. Materials in contact with the LED chip (silicone on top and lateral, EpoTek and Au pads at the bottom) have the same temperature at the interface, however, the thermal conductivities and capacitances of the applied materials (table S1) lead to different heat flows towards the top, side and bottom of the oCI. Consequently, the surface temperatures

in a worst-case scenario with $I_{LED} = 38.9$ mA and $t_{on} = 10$ ms are 74.1°C, 54.7°C, and 50°C at the oCI top, side and bottom, respectively, are not compatible with in vivo applications. However, the thermal conductivity of the surrounding aqueous media distributes the thermal energy across a larger volume. This results in a temperature increase smaller than 1 K at distances of 104 μ m above, 71 μ m to the side of and 67 μ m below the oCI surface even at these extreme operational parameters.

Virus purification and postnatal viral transduction of rats and Mongolian gerbils.

Virus purification: AAV-PHP.B virus used in this study for transduction of SGNs of P6-P7 wildtype rat or gerbil pups was generated as previously described (1, 2). In brief, HEK293T cells were triple transfected with pHelper plasmid (TaKaRa/Clontech), trans-plasmid providing viral capsid PHP.B (generous gift from Ben Deverman and Viviana Gradinaru, Caltech, USA) and cis-plasmid providing channelrhodopsin CatCh under human synapsin promoter. Viral particles were harvested 120 h after transfection and purified using iodixanol (Optiprep, Axix Shield). Finally, the particles were concentrated using Amicon filters (EMD, UFC910024) and the titer was measured using AAV titration kit (TaKaRa/Clontech). Viral stocks were kept at -80°C until injection.

Postnatal viral transduction: Injections were performed using micropipettes (20 μ m diameter) pulled from quartz capillaries on a P-2000 laser puller (Sutter Instruments) connected to a pressure microinjector (100 – 125 PSI, PLI-100 pico-injector, Harvard Apparatus) as previously described (1, 3). In brief, under general isoflurane anesthesia and local analgesia achieved by xylocaine, the left ear was approached via a dorsal incision and the round window membrane was identified and gently punctured using a borosilicate micropipette that was kept in place to inject approximately 1–1.5 μ l of AAV-PHP.B_hSyn_CatCh-EYFP_WPRE_bGH (8.72×10^{12} GC/ml). After virus application, the tissue above the injection site was repositioned, the wound was sutured, and buprenorphine (0.1 mg/kg) was applied for appropriate analgesia. In addition, the injection site was treated with Zincojocol WDT cream (WDT, Germany) to speed-up the healing processes and to reduce the rejection of pups from the parents after they were put back to the cage previously supplemented with extra nutrients (Nutri-plus cat, Virbac). Recovery of the animals was then tracked regularly. In all experiments, rats and gerbils were randomly selected for injection. Animals were then kept in a 12-h light/dark cycle, with access to food and water ad libitum.

Surgery.

For in vivo experiments, animals were anesthetized with isoflurane (4 % at 1 l/min for induction, 1–2 % at 0.4 l/min for maintenance) and appropriate analgesia was obtained with subdermal injection of buprenorphine and carprofen. The animals were then placed onto a custom-designed heating blanket to maintain physiological body temperature on a vibration isolating table in a sound-proof chamber. Access to the left cochlea for stimulation was achieved by a retroauricular approach. Optical stimulation was performed through the round window or via a cochleostomy.

For thermal characterization during LED stimulation, a commercial thermometer (HH506A, Omega Engineering) with a K-type temperature probe ($\pm 0.05\%$ rdg + 0.3°C) was placed in the round window while the oCI was inserted into the cochlea via cochleostomy. The temperature

was recorded every minute and normalized to the averaged baseline measurement of each animal. For morphological studies, we euthanized the animals with isoflurane followed by cervical dislocation and cochleae were explanted and fixed with 4% paraformaldehyde in PBS for 1h.

Immunohistochemistry.

Cochlear cryosections: Sample preparation and immunohistochemical staining were performed as described previously (4). Cochleae of both sides were removed from the temporal bone immediately after sacrificing the animal and fixed in 4 % paraformaldehyde (PFA) in PBS for 1 h. Cochleae were decalcified in Ethylenediaminetetraacetic acid (EDTA; 0.12 M for 3–4 days) and subsequently cryosectioned. Immunolabeling for parvalbumin and CatCh-EYFP was performed on 16 μ m thin cryosections with anti-GFP (1:500, chicken, Cat. #ab13970, Abcam) and anti-parvalbumin (1:300, guinea pig, Cat. #195004, Synaptic Systems) antibodies, respectively, and appropriate secondary antibodies goat anti-guinea-pig 568 (1:200, Cat. #A11075, Thermo Fisher Scientific) and goat anti-chicken Alexa Fluor 488 (1:200, Cat. #A11039, MoBiTec). Finally, specimens were mounted using Mowiol mounting medium and images were acquired using an Abberior Instruments Expert Line microscope in confocal mode (for rat) or a ZEISS LSM800 microscope (for gerbil) using a 20 $\times$ oil immersion objective and a 20 $\times$ air objective, respectively. Images were processed for display in ImageJ (45) and assembled in Adobe Illustrator.

Organ of Corti whole-mount: Sample preparation and immunohistochemical staining were performed as described previously (4). In brief, after sacrificing, cochleae from adult control and pharmacologically-deafened rats were dissected in phosphate-buffered saline (PBS) and fixed with 4% formaldehyde-containing PBS for 1 h on ice. Subsequently, cochleae were decalcified in 0.12 M EDTA solution for 10–14 days at 4°C, prior to microdissection of the organ of Corti and immunolabelling. Here, specimens were incubated with goat serum dilution buffer (composed of: 16% normal goat serum, 450 mM NaCl, 0.3% Triton X-100, 20 mM phosphate buffer, pH 7.4) to block reactive but unspecific binding sites (1 h at room temperature) and then incubated overnight at 4°C with a primary antibody targeting the cytosolic Ca^{2+} buffer calretinin (1:300; Swant, CR7697). For detection, a secondary Alexa Fluor 568-conjugated goat anti-rabbit secondary antibody (Thermo Fisher Scientific, Cat. #A11011) was used to visualize calretinin, while an Atto647N-conjugated phalloidin (Merck; Cat. #65906) served as a context marker to label f-actin within the tissue (e.g. actin is highly enriched in hair cell cuticulate plates, stereocilia and supporting cells). Finally, specimens were mounted using Mowiol mounting medium and imaged either on an (i) Axio Imager M2 System (5 $\times$ objective, Zeiss) controlled by a Neurolucida system (Version 11, MBF Bioscience) to obtain larger overviews covering the entire apical to middle turn, or (ii) a Leica TCS SP5 laser-scanning confocal microscope with a 1.4 NA 63 $\times$ oil immersion objective and HeNe 561 nm and 633 nm excitation laser lines. Images were then processed for display in ImageJ (45) and assembled in Adobe Illustrator.

X-ray tomography.

Positioning of the oCI was confirmed with phase-contrast tomography at a compact laboratory x-ray source essentially as previously described (16). The custom-built cone-beam in-

line tomography instrument equipped with a liquid-metal x-ray source (JXS D2, Excillum) operated at 70 kV acceleration voltage was combined with a 20 μm LuAG-scintillator-based fiber-coupled CCD detector (6.5 μm pixel size; C12849-102U; Hamamatsu). Fast Fourier-based phase reconstruction was used to calculate the image volume. Reconstruction was performed using the ASTRA toolbox (46, 47). Segmentation and 3D visualization of the reconstructed volume was performed with Avizo 3D 9 (Visualization Sciences Group, FEI Company). Automatic histogram-based segmentation was used to visualize bony structures. The basilar membrane, Rosenthal's canal, scala vestibuli, scala tympani and oCI were traced with semiautomatic segmentation. Insertion depth was measured with a fitted spline curve to markers along the oCI.

Shuttle box behavior.

For behavioral testing, a custom-built shuttle box located in a sound attenuated chamber (75×50×100 cm (W×D×H); IAC Acoustics) was used, as described before (16). The shuttle box consists of two platforms (25×25 cm; each assembled from 16 metal bars of 4 mm diameter, 15 mm spacing center to center), separated by a hurdle (4 cm height) and surrounded by plastic tubes (2.5 cm diameter, spaced 3.5 cm center to center; 35 cm height). Both platforms were mounted on an individual set of metal springs (4 per platform) and equipped with acceleration sensors (LIS344ALH; STMicroelectronics) to determine the position of the animal. Generation and presentation of stimuli as well as data acquisition was done using custom-written MATLAB (The MathWorks, Inc) scripts actuating NI-DAQ cards (National Instruments Corporation) combined with custom build hardware for signal attenuation and amplification. Acoustic stimuli were delivered using a loudspeaker (ScanSpeak, Avisoft Bioacoustics) centered over the shuttle box and mounted to the ceiling of the chamber. For behavioral training, animals were placed in the shuttle box and given an adaptation time of 5 minutes before stimuli were presented. For acoustic or optical (via the oCI) stimulation, trains of 12 clicks or 50 Hz light pulse (10 ms) within 250 ms were applied, which was repeated with rate of 2 Hz during a 6 second window (70 trials per session with an inter-trial-interval randomized between 14 and 24 seconds). Upon perception of a stimulus the animal was expected to cross the hurdle of the shuttle box. If the animal crossed the hurdle within the response window, the trial was considered a hit and the stimulus was switched off. If the animal did not cross the hurdle in time, an aversive electric stimulus (0.05–1 mA) was administered to the animal's feet via the metal bars of the platform until the animal crossed the hurdle (or for a maximum of 6 seconds) and the trial was considered a miss. Per session, 20 sham trials in which no stimulus was presented and accordingly no consequences for the reaction of the animal existed were randomly intermixed with regular target trials in order to determine the baseline activity of the animal. For naïve oCI implanted rats sessions with three different stimuli were performed to rule out that animals forget the prior learned induced avoidance behavior: in each session ($n = 3$) 30 acoustic, 20 optical and 20 sham trials were presented whereas rats did only respond to acoustic but did not show avoidance behavior after optical stimulation. Sessions with preprogrammed stimulation (Fig. 7E right panel) as well as sessions using acoustic cues for stimulus triggering (Fig. 7F left panel) were performed [detailed technological description and characterization in (35)]. To rule out that the avoidance behavior of the animals in the sessions when acoustic cues were used for stimulus triggering (movie S3) was caused by remaining or recovered hearing ability, aABRs were measured in these animals subsequent to these sessions. Only animals without aABRs at 110 dB

clicks as well as pure tones were included in the analysis. In the last session of the AAV-transduced rats, the sound processor was turned off in order to ensure that animals did not use any cues (e.g. visual) other than optogenetic stimulation to solve the task (Fig. 7F right panel).

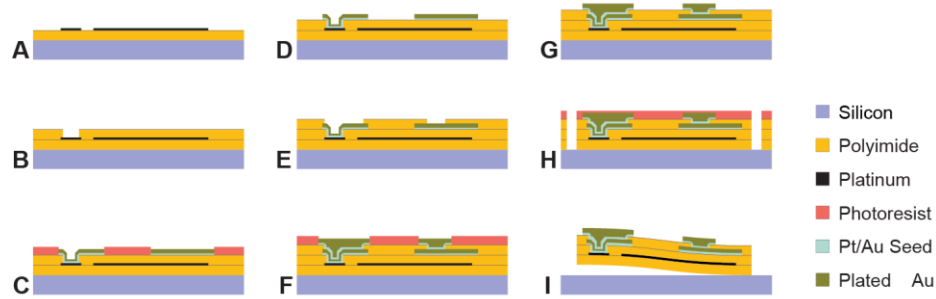


Fig. S1. Fabrication of the highly flexible oCI substrates.

(A) Spin coating of first PI layer on Si handle wafer and lift-off patterning of Pt metal lines forming the temperature sensing element in the first metallization layer. (B) Spin coating of second PI layer, RIE patterning to open vias using photoresist mask. (C) Deposition of metal seed layer and patterned Au electroplating of the second metallization using a second photoresist mask. (D) Resist strip and seed layer etch. (E) Spin coating of the third PI layer and via opening using a third photoresist mask and RIE. (F) Metal seed layer deposition and patterned Au electroplating of the third metallization. (G) Resist strip and seed layer etch. (H) oCI probe patterning using a fourth photoresist mask and RIE. (I) Photoresist strip and peeling the patterned optical probes off the handle wafer.

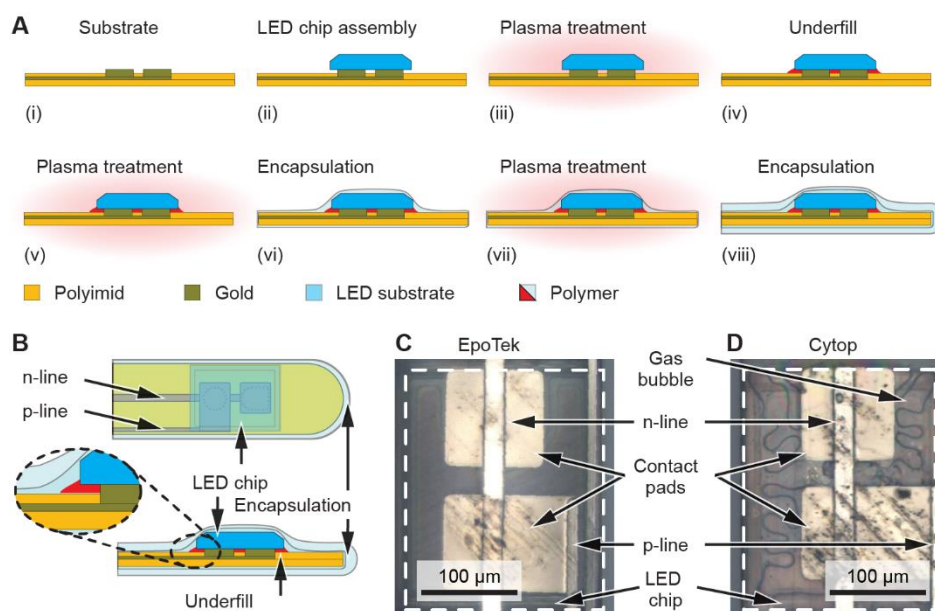


Fig. S2. Assembly and passivation of oCIs.

(A) Individual LED assembly on the (i) flexible PI substrate using (ii) flip-chip bonding and (iii) surface cleaning and adhesion promotion using argon plasma treatment. (iv) LED underfill with EPO-TEK 301-2. (v) Argon plasma treatment for adhesion promotion and surface cleaning. (vi) Inner Cytop encapsulation. (vii) Oxygen plasma treatment for adhesion promotion and surface cleaning. (viii) Outer silicone encapsulation. (B) Schematic of an LED on a PI substrate illustrating the underfill and exposed materials. (C) Microscopic image of perfectly underfilled LED by using solvent-free EPO-TEK before applying the Cytop- and silicone encapsulation layers. (D) Underfill grade of 77 % by applying Cytop showing voids in the Cytop layer.

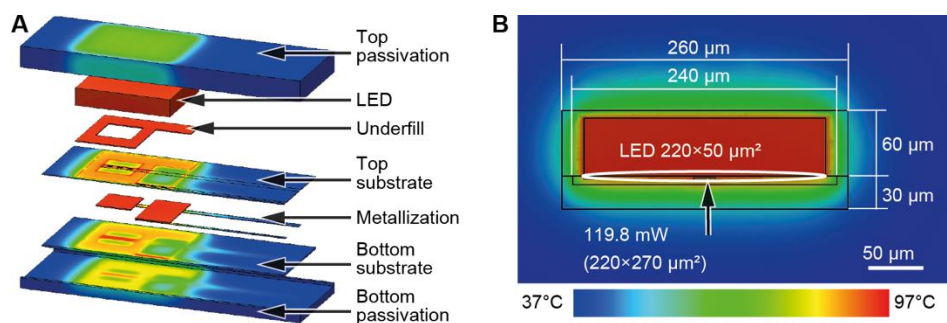


Fig. S3. Thermal simulation of an oCI.

(A) Exploded false-color schematic of the simulation model with its essential components, i.e. silicone passivation, SiC LED, EPO-TEK underfill, PI substrates, and Au metallization. The coloring indicates the temperature profiles on the surfaces of the individual components. (B) False-color cross-sectional view of the simulation model with key dimensions. The illustrated temperature profile indicates a high LED chip temperature of 97°C, but as well a fast temperature decay within the water cube.

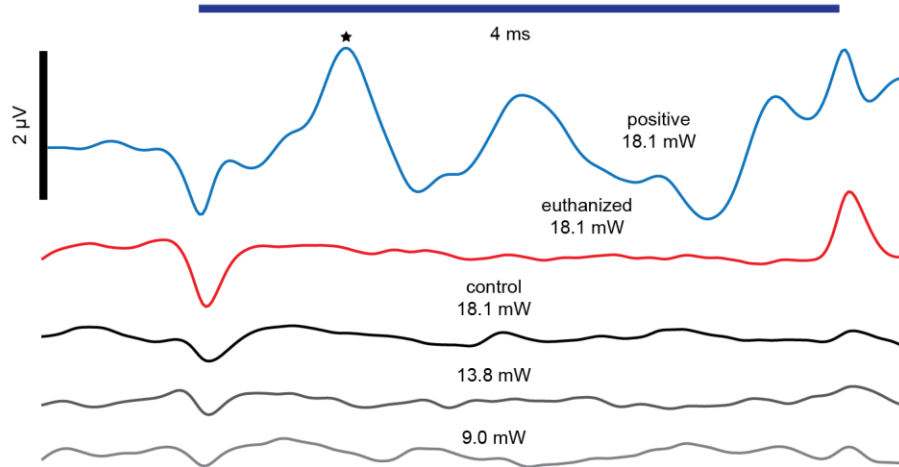


Fig. S4. oCIs do not evoke potentials in littermate control rats lacking ChR2 or in ChR2-positive rats after euthanasia.

Upper two traces show stimulus-locked average responses to intracochlear LED-based oCI stimulation in a ChR2-positive rat before (blue: clear oABR, p1 labeled by an asterisk, 18.1 mW, 10 Hz stimulus rate, 4 ms stimulus duration) and after euthanizing (red, no oABR, only artifacts) for reference. Lower three traces were obtained from a ChR2-negative littermate rat at different light intensities (9.0 to 18.1 mW, 10 Hz stimulation rate, 4 ms stimulation duration). The oCI was placed in the middle cochlear turn via a cochleostomy in all cases. Stimulus is displayed as blue bar.

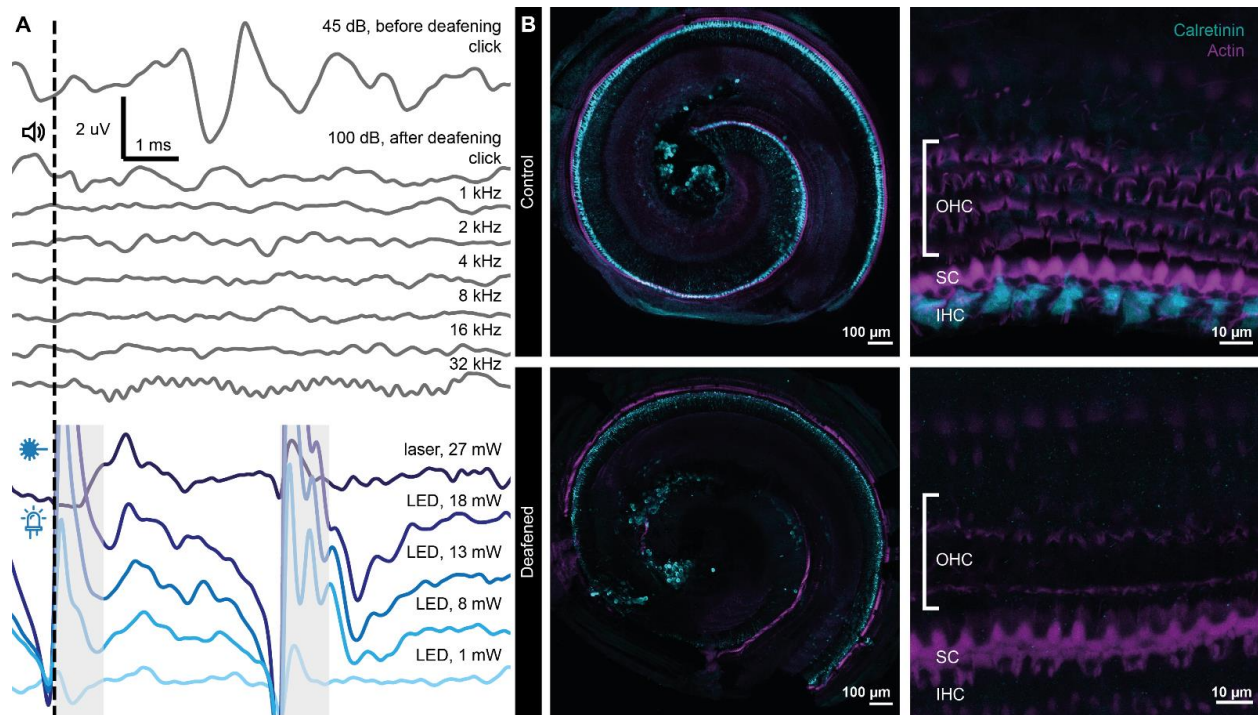


Fig. S5. Kanamycin-induced deafness in rats.

(A) Acoustically evoked auditory brainstem recordings (ABRs) were performed in adult rats prior and after application of kanamycin to the cochlea. Thresholds were above 100 dB for clicks and pure tones from 1–32 kHz after deafening. Optically-evoked ABRs (4 ms, 10 Hz, 1000 repetitions) could be elicited via fiber-coupled laser and individual LEDs. Artifacts during LED stimulation were in the range of 20 μ V and clipped (grey area). (B) Histological verification of inner hair cell (IHC) loss upon deafening. Overview of the organ of Corti in a control animal (top left) and a kanamycin deafened animal (bottom left). Atto647N-conjugated phalloidin was used to stain actin (purple) prominently expressed in hair cells and supporting cells (SC), and calretinin was used to stain spiral ganglion neurons (SGNs) and IHCs (cyan). Note the lack of IHCs in the deafened animals. The spiral ganglion afferents are still present in the deafened animals. Right: Magnification of the hair cell regions.

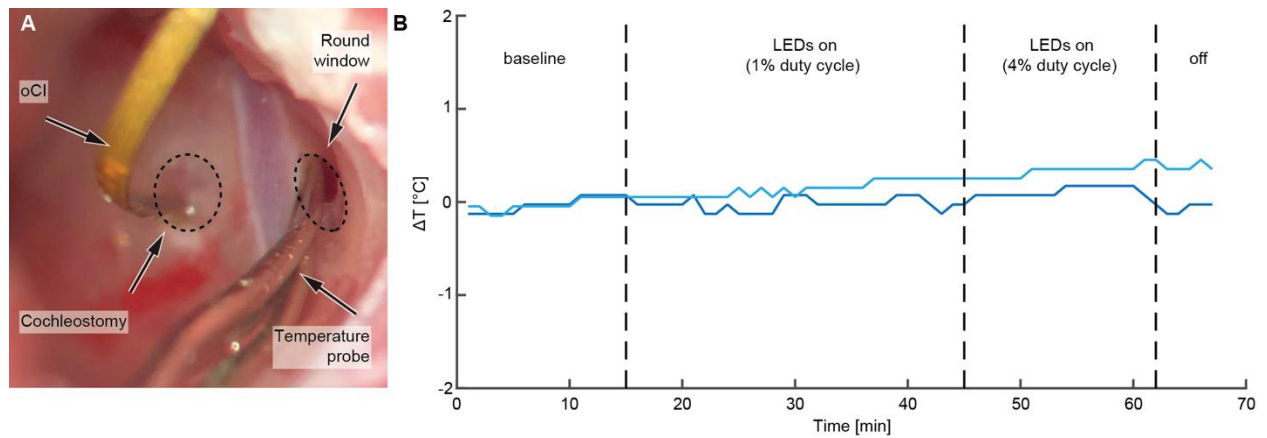


Fig. S6. Intracochlear temperature measurements in rats.

(A) Photograph showing the oCI inserted into the cochlea via cochleostomy in the first turn of adult rats ($n = 2$). The commercial temperature probe was inserted into the round window and recorded every minute. (B) The temperature recordings were normalized to the baseline measurement and displayed as temperature change. LED stimulation (6 LEDs, 10 mA, 10 Hz, 1 ms/4ms) was altered between 1% and 4% duty cycles. The accuracy value of the thermometer at 39°C is $\pm 0.3^\circ\text{C}$. The measured temperature shifts were below the accuracy value. The average temperature shift at 1% and 4% duty cycle is 0.1°C and 0.2°C , respectively.

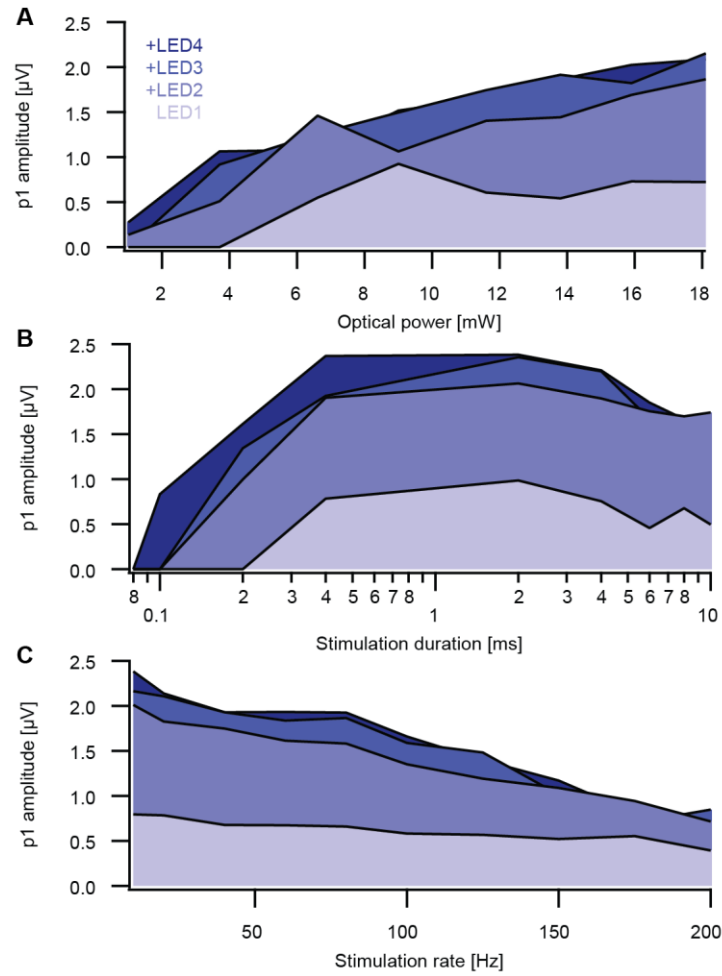


Fig. S7. Additivity of LEDs in eliciting oABRs in rats.

The oCI was placed on the middle cochlear turn via cochleostomy. oABR amplitude of p1 recorded from one rat with subsequent addition of up to four LEDs (light to dark blue) as a function of radiant flux (A), stimulus duration (B) and stimulation rate (C). LED 1 was inserted for about 3 mm, LED 4 was at the bony edge of the cochleostomy, which might explain the relatively small gain of LED 4.

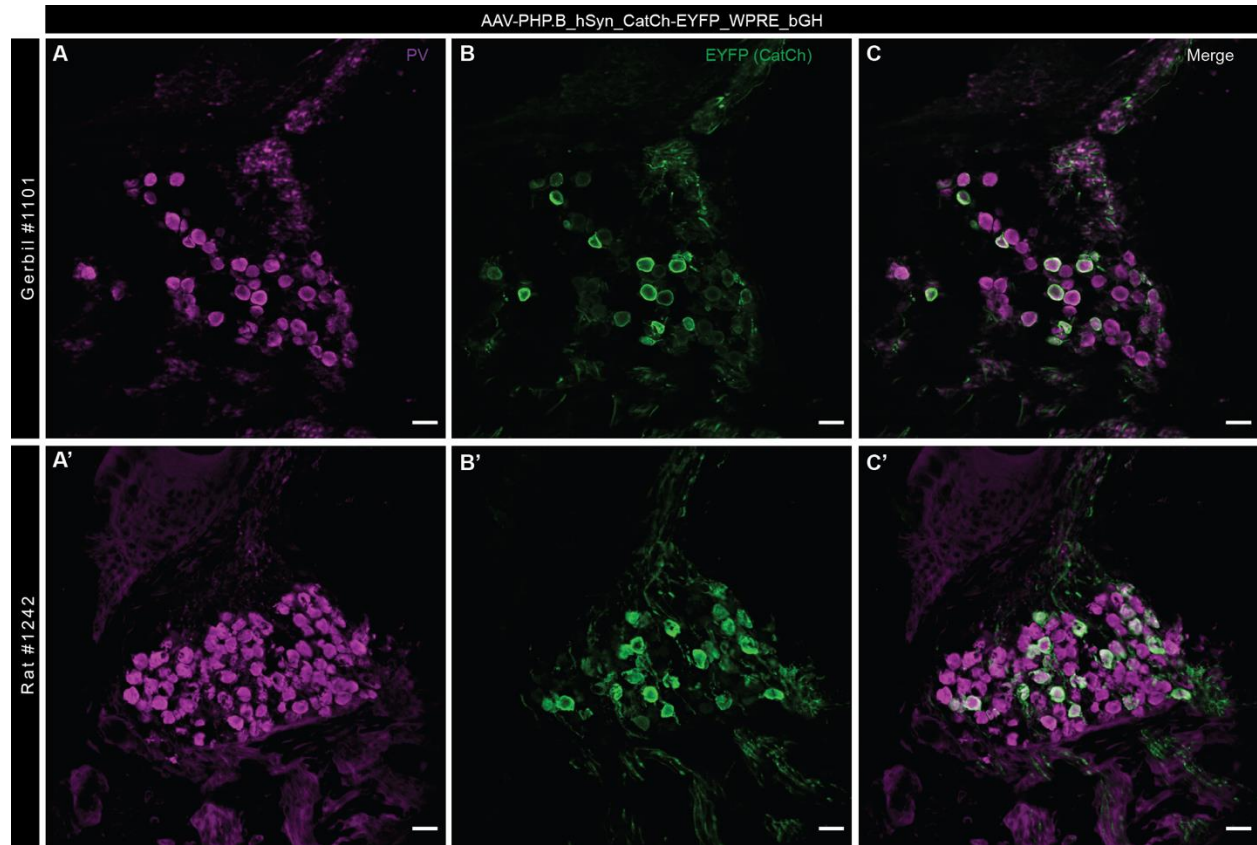


Fig. S8. Early postnatal transduction of gerbil and rat cochlea.

Parvalbumin (PV) immunostaining of SGNs in P6 CatCh transduced gerbil (**A**) and rat (**A'**). EYFP (CatCh) immunostaining in gerbil (**B**) and rat (**B'**). (**C**) and (**C'**) Merge immunostaining of PV positive and EYFP positive (CatCh) SGNs. Scale bar 20 μ m.

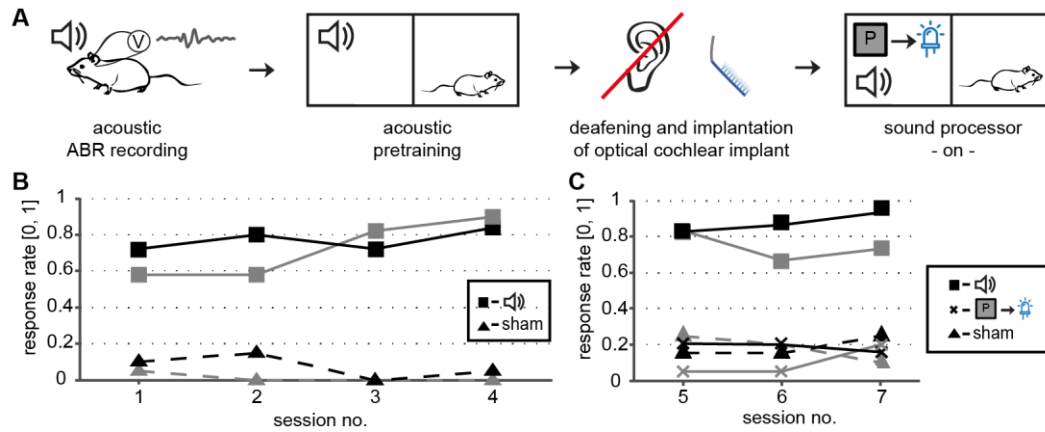


Fig. S9. oCIs do not cue avoidance behavior in naïve rats.

(A) Experimental workflow. Acoustic ABRs were recorded in naïve adult rats ($n = 2$) and animals were trained in the shuttle box four times using acoustic stimulation. Rats were deafened on the left side by intracochlear injections of kanamycin solution. In the same surgery rats were implanted with an optical cochlear implant also on the left side. Subsequently, shuttle box experiments with three paradigms (acoustic, optical, sham) were performed. (B and C) Different animals are indicated by colors (black, grey) while different stimuli are indicated by symbols (acoustic-square; optical-cross; sham-triangle). (B) Response rates in shuttle box sessions during initial acoustic training. (C) Response rate in sessions with either acoustic, optical or sham (no) stimulation.

Table S1. Thermal simulation material properties. Material properties were used to simulate the temperature distribution using SolidWorks Simulation.

	Material	Thermal conductivity [W/mK]	Specific heat capacity [J/kgK]
LED	4H-SiC	370	690
Substrate	Polyimide	0.29	1130
Metallization	Gold	300	130
Underfill	EpoTek 301	0.223	1630
Passivation	Silicone	0.2256	1386
Surrounding	Water	0.61	4200

Data file S1. Raw data for graphs containing an n lower than 20. Provided as a separate Excel file.

Movie S1. Visualization of implanted cochlea by high-resolution x-ray tomography. 3D-visualization of an explanted rat cochlea obtained by x-ray phase contrast tomography first displayed as raw x-ray sections and volume rendering representing pixel values in grey scale. Segmented model reveals LEDs (blue), basilar membrane (green) scala tympani (turquoise) and Rosenthal's canal (purple). Towards the end the camera follows the path of the oCI into the scala tympani via the round window.

Movie S2. Visualization of oCI stimulation in the explanted rat cochlea. Explanted rat cochlea submerged in thiodiethanol (to render the bone translucent) with fully implanted 10 LED oCI in the basal turn via the round window. LEDs were activated via fast Fourier transformed audio track signal. Please note that due to the low frame rate of the camera some μ LED pulses were not captured.

Movie S3. Video of parts of a shuttle box session in a deaf, AAV-injected rat with the complete oCI system. Corresponding aABRs for this animal are shown in Fig. 7. Sound triggers optical stimulation of the cochlea via sound processor so that animal can perform avoidance behavior (change of the sides during stimulation). Video includes sound (acoustic click stimulation also indicated by a speaker symbol appearing in the corresponding frames).