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Optogenetic stimulation of the auditory pathway for research and future prosthetics

Tobias Moser[1,2,3]

Sound is encoded by spiral ganglion neurons (SGNs) in the hearing organ, the cochlea, with great temporal, spectral and intensity resolution. When hearing fails, electric stimulation by implanted prostheses can partially restore hearing. Optical stimulation promises a fundamental advance of hearing restoration over electric prostheses since light can be conveniently focused and hence might dramatically improve frequency resolution of sound encoding. Combining optogenetic manipulation of neurons with innovative optical stimulation technology promises versatile spatiotemporal stimulation patterns in the auditory system. Therefore, using optical stimulation of SGNs also has great potential for auditory research. Here, I review recent progress in optogenetic stimulation of the auditory system and its potential for future application in research and hearing restoration.

Addresses
[1] Institute for Auditory Neuroscience and Collaborative Research Center 889, University Medical Center Göttingen, Göttingen, Germany
[2] Bernstein Center for Computational Neuroscience, University of Göttingen, Göttingen, Germany
[3] Center for Nanoscale Microscopy and Molecular Physiology of the Brain, University of Göttingen, Göttingen, Germany

Corresponding author: Moser, Tobias (tmoser@gwdg.de)

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Why consider optical stimulation of auditory neurons for research?

Acoustic and electric stimulation of the auditory system has been very successfully employed to elucidate how hearing works. Still there are shortcomings of either approach. Acoustic stimulation inevitably is confounded by the properties of cochlear micromechanics that limit the spectral resolution of sound encoding and the independence of SGN activation at different position along the tonotopic axis of the cochlea in a stimulus-level-dependent manner. Therefore, investigating the spectral bandwidth of stimulus-specific adaptation in the auditory cortex, for example, has remained challenging [1]. Likewise, cochlear micromechanics limits the frequency resolution for studying spectral integration by the central auditory system e.g. in processing the localization of sound. Furthermore, efforts to decipher the processing of complex acoustic stimuli by the brain such as amplitude-modulated sounds have been limited by cochlear mechanisms [2]. Focused optical stimulation of SGNs promises to overcome this limitation, provided the other attributes of physiological sound encoding such as temporal fidelity can be achieved. Given appropriate optical stimulation technology, the method promises flexibly selectable spatiotemporal patterns of excitation in the cochlea and central auditory pathway that offer plentiful opportunities of research on auditory function and dysfunction.

Why developing optical stimulation for improved hearing restoration?

Approximately 360 million people–5% of the world's population — suffer from disabling hearing impairment (HI) [3], commonly causing social isolation, depression, and reduction in professional capabilities. So far despite major research efforts, a causal treatment based on pharmacology, gene therapy or stem cells is not yet available for its most common form: sensorineural HI that is typically caused by cochlear dysfunction or degeneration and often involves the loss of sensory hair cells. Hearing aids and auditory implants, cochlear implants (CIs) and auditory brainstem implants (ABIs), represent the state-of-the-art approaches for partial restoration of auditory function and are likely to remain key means for alleviating sensorineural HI during the coming decades. The CI bypasses cochlear dysfunction via direct electric stimulation of SGNs and is the most successful neuroprosthesis employed by more than 300,000 users. CIs enable open speech comprehension in most users [4,5,6•] while the benefit from aiding with ABIs that electrically stimulate the cochlear nucleus is more heterogeneous [7]. Moreover, auditory implants have become a major tool in auditory research [8]. However, the use of current clinical CIs has limitations arising from the wide spread of current around each electrode contact [9] which leads to channel-crosstalk [10]. This limits the number of useful frequency channels to less than ten [11]. As a result, CI users suffer from poor comprehension of speech in noisy environments and typically cannot appreciate music. Increasing the frequency and intensity resolution of auditory coding is a central objective for improving the CI. Research toward better frequency resolution of electrical CIs includes multipolar stimulation [12], intraneural electrodes [13•], efforts to

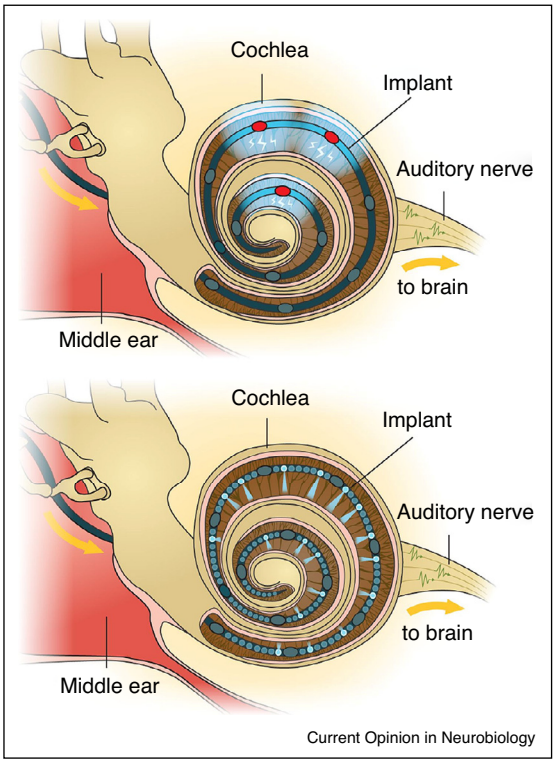
achieve outgrowth of neurites toward CI contacts (e.g. ref [14•]) and most recently optical stimulation [15••,16••].

Optical stimulation strategies

Optical stimulation of SGNs is a novel approach that promises a dramatic increase of the frequency resolution of CI-coding via more spatially confined SGN activation [15••,16••] (Figure 1). In addition, activation of smaller populations of neurons can also enhance the dynamic range of coding e.g. by varying the number of activated neighboring stimulation channels [17].

Currently, two optical strategies for driving auditory neurons are pursued: infrared neural stimulation and optogenetic stimulation. Richter *et al.* have pioneered infrared neural stimulation [18] and characterized energy requirement [19•], temporal fidelity [20], spatial spread of excitation [15••,21]. The reported spatial spread of cochlear excitation was much better than for electrical stimulation, but the energy requirement [15••] greatly exceeded that in clinical implants [22]. Recently, the concept has been challenged by the observations that optoacoustic stimulation of the cochlea by strong laser pulses may confound the results [24•,25••], that infrared laser stimulation does not trigger auditory activity in completely deafened cochleae [25••] and that the postulated membrane-bound optothermic mechanism of depolarization, when characterized in cultured cells [23•], seems to require more energy than activation of the auditory pathway *in vivo*. More work is required to further evaluate the mechanisms and feasibility of direct infrared neural stimulation of the auditory nerve. Less light is required for optogenetic stimulation (see below) that involves the expression of photosensitive proteins to enable optical control of cells via light-driven ionotropic or metabotropic cellular signaling [26].

Figure 1



Electrical versus optical SGN stimulation. **Top:** In electrical CIs usually 8-24 electrode contacts (blue or red) are used to stimulate SGNs by charge-neutral biphasic stimuli in a monopolar configuration (red contacts). Current spread leads to activation of a large population of neurons along the spiral tonotopic axis by any active contact (blue halo), thereby limiting the frequency resolution of electrical coding. **Bottom:** optical stimulation e.g. light emitted from microscale light emitting diodes (μLEDs) focused by lenses or emitted from waveguide arrays promises spatially confined activation of SGNs allowing for a higher number of independent stimulation channels and improving the frequency and intensity resolution of sound coding.

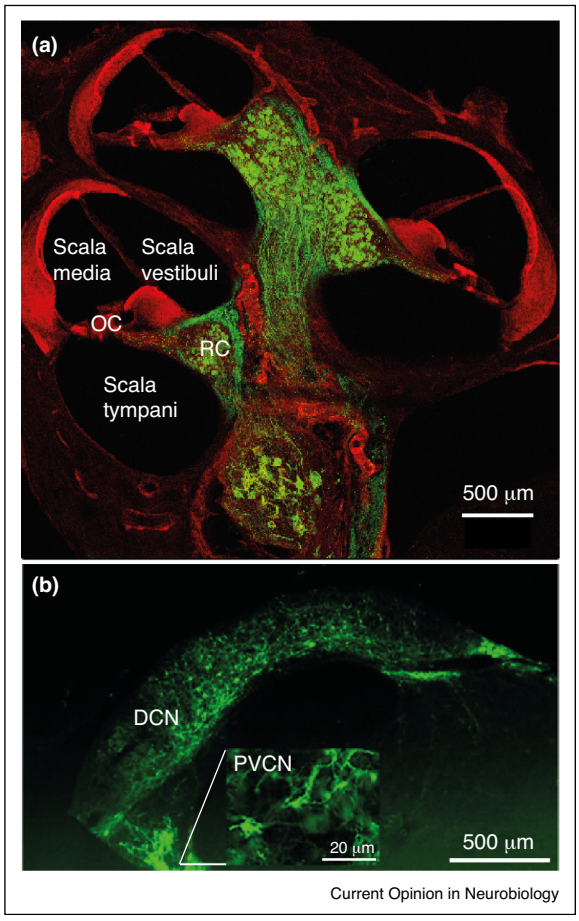
Optogenetic stimulation of the auditory system

Starting with the discovery and characterization of channelrhodopsins 1 [27•] and 2 [28•], optogenetics has revolutionized the life sciences and is in rapid development with various applications and advances such as the engineering of improved opsins and vectors [29]. The first applications of optogenetics to auditory research employed channelrhodopsin 2 (ChR2) for enabling blue light-driven activity of SGNs, cochlear nucleus and auditory cortex neurons [16••,30,31••] or halorhodopsin [32•] for inhibiting cochlear nucleus neurons [31••]. Optogenetic stimulation of the ascending auditory pathway was demonstrated by electrophysiology at the single neuron and neuronal population levels [16••,31••] even in mouse models of human deafness [16••]. Whether and how the auditory cortex is activated and a sensory percept is generated upon optogenetic stimulation of the ascending auditory pathway has yet to be determined. Both germline mutagenesis and viral transduction established sufficient levels of ChR2 expression in auditory neurons for depolarization by low light intensities (few mW/mm^2). Light requirements for cochlear stimulation were 1-2 orders of magnitude lower than reported for infrared cochlear stimulation [16••]. The lower light requirement of optogenetics also makes the approach compatible with several microscale optical technologies such as microscale light emitting diodes (μLEDs) and vertical cavity surface emitting laser (VCSELs).

Strategies for opsin expression in the auditory system

Transgenic mice [33•] and rats [34] with neuronal ChR2 expression under Thy1.2 promoter (Figure 2) provide convenient access to optogenetic stimulation of the auditory pathway [16••]. However, potential effects of activating non-auditory neurons need to be considered. Employing conditional mutants [35] and mouse lines selectively expressing Cre-recombinase or opsins in auditory neurons will be an important next step toward specific optogenetic manipulation of the auditory system.

Figure 2



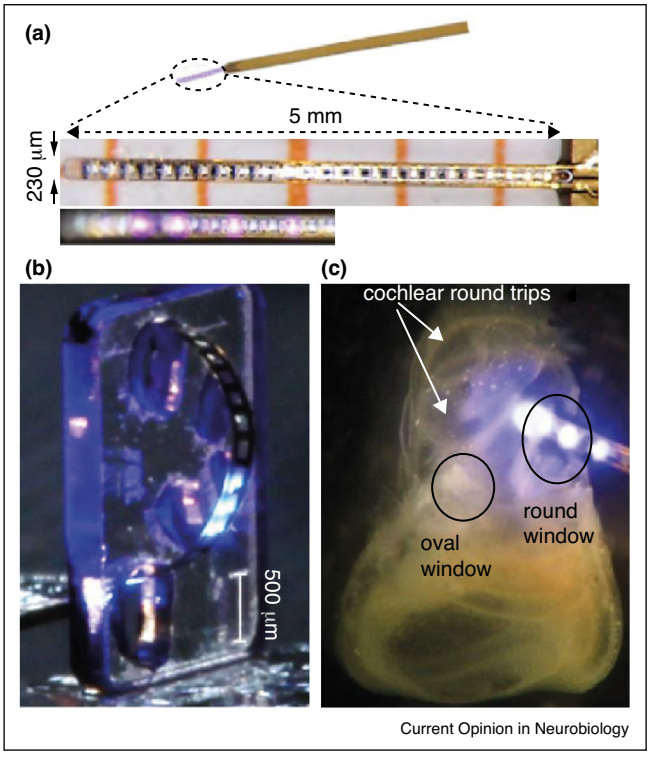
Expression of ChR2 in auditory neurons. (A) ChR2-YFP expression in SGNs of a transgenic mouse [32•] in a section of an entire mouse cochlea following GFP immunolabeling (green) and phalloidin-AF-568 labeling of actin (red). The section roughly hit the center of the cochlea and reveals much of the spiral ganglion in Rosenthal's canal (RC). Moreover, the scalae of the cochlea are easily visible, whereby the sensory organ of Corti (OC) rests in-between Scala media and Scala tympani. Scala media and vestibuli are separated by Reissner's membrane (thin red structure). Image: courtesy of Dr. Hernandez. **(B)** Horizontal brainstem section following AAV injection and immunolabeling for GFP: expression of ChR2 in the cochlear nucleus. DCN: dorsal cochlear nucleus, PVCN: posteroventral cochlear nucleus. Modified after reference [31••].

Virus-mediated optogenetics offers flexibility for testing various ChR variants, some specificity by the choice of virus capsid, promoter of the transgene as well as by the site and time of virus application, and is the most promising approach for using optogenetics in auditory research on species not available to germline mutagenesis as well as for future clinical translation. Among the various viruses tested, adeno-associated virus (AAV) gave the best expression in SGNs [16••] and were also successfully used for optogenetic stimulation of the cochlear nucleus [31••]. AAV, despite not integrating into the genome, provided long-lasting opsin expression upon a single injection (up to at least 18 months in the cochlear nucleus, [31••]) and did not cause any obvious signs of neuronal toxicity or loss in the cochlea or cochlear nucleus [16••,31••]. Neither germline-transmitted nor virus-mediated transgenic expression of ChR2 in auditory neurons compromised acoustic hearing [16••,31••]. While AAV-mediated ChR2 expression in the cochlear nucleus was achieved by postnatal virus injection [31••], so far a transuterine approach to the mouse embryonic ear [36] has been employed for expressing the ChR2 variant CatCh [37•] in SGNs [16••]. The latter approach offered the advantage of manipulating several animals per surgery. It also provided insight into the minimal fraction of ChR-positive SGNs required for obtaining an optogenetic population response of the auditory pathway (40%) but, within the specific protocol, failed to express ChR in the apex of the cochlea. Optimization of AAVs for penetration and cell-type specific transduction in a given species is an active area of research providing tailored and improved reagents [38,39•,40]. AAV-mediated gene transfer to the human eye has been proven to be efficient and safe in clinical trials following single-term subretinal injection [41]. Future work toward clinical translation should also consider nonviral transduction such as electroporation by CI electrodes [14•]. Moreover, considering the case of deafness with major loss of SGNs, that may in the future be served by grafting stem cell-derived otic neural precursor cells to regenerate functionally connected SGNs [42], the specific and confined optical stimulation of ChR-expressing grafted cells could help evaluating the function of grafted cells and provide a one step procedure for regenerating SGN and CI with improved frequency resolution.

Optical stimulation strategies tailored to the auditory system

Optical stimulation promises stimulation with improved spatial and cellular selectivity. It may also enable closed-loop stimulation, because of less interference with electrical recordings, and thereby e.g. facilitate the fitting of a cochlear implant. Recent progress in optoelectronics and material science has prepared the ground for devising new means of stimulating the cochlea and the central auditory pathway. Intracochlear stimulation needs to consider cochlear morphology for the particular species. X-ray tomography has been instrumental to gain such information with high resolution [16••,43•,59]. Implantable optical stimulation can consider 'active' and 'passive' solutions, which either use implanted optoelectronics to generate light inside the organ or guide light into the tissue from an outside source. So far waveguides coupled to lasers and closely apposed or implanted μLEDs have been employed to demonstrate feasibility of single channel stimulation with passive and active optical implants in auditory optogenetics [16••,31••]. Passive solutions, such as single waveguide or waveguide arrays, offer the advantage of good tissue compatibility and stability. Moreover, this approach should be least prone to electrical recording artifacts since light source and tissue are well separated. Dependent on whether the application

Figure 3



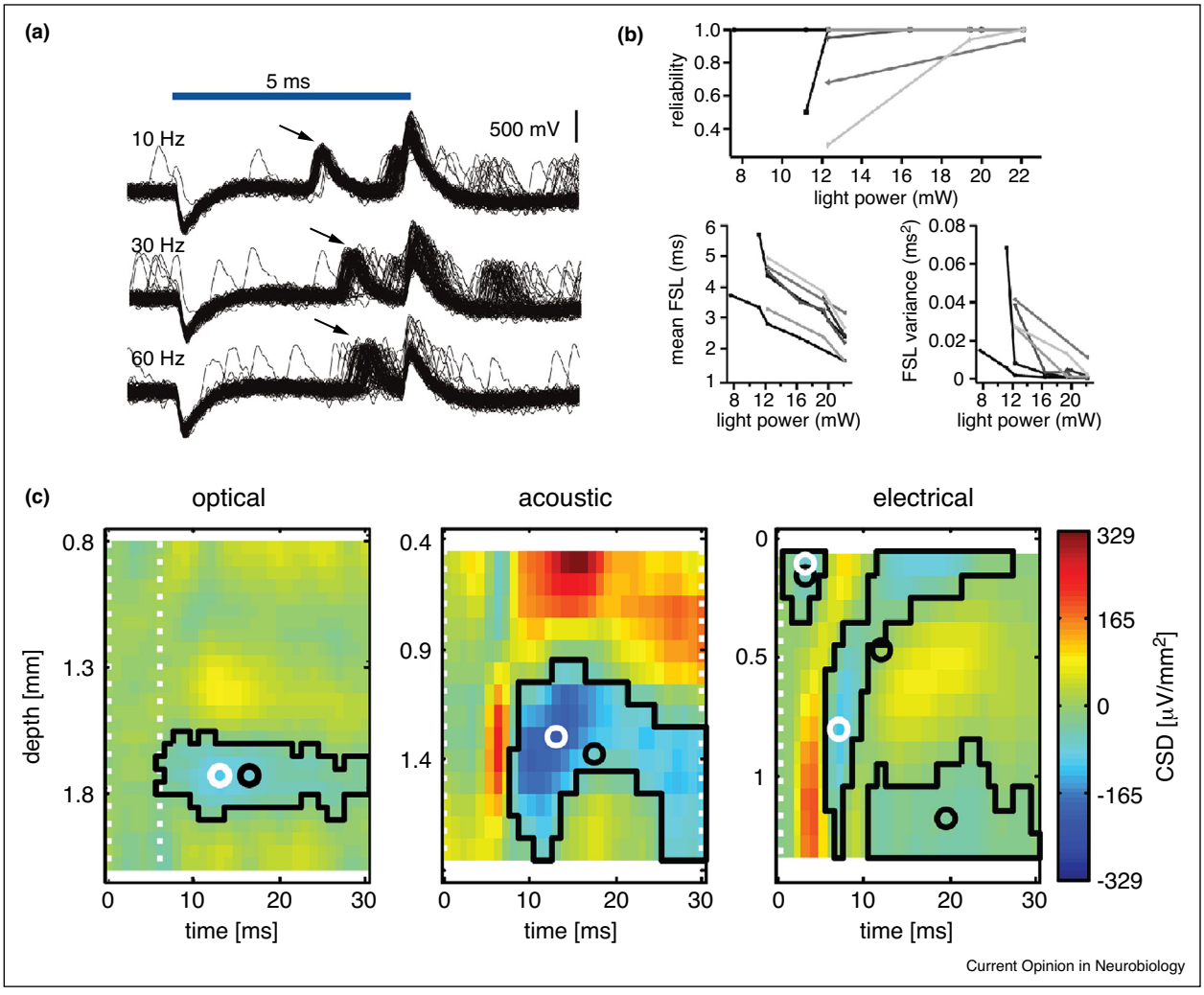
Technology development for optical multichannel stimulation. (A) shows a µLED-array on flexible substrate (high magnification) mounted to a flexible circuit board with 405 nm emitting LEDs (lowest panel, taken from [44]). **(B)** Functional bending test with a flexible µLED-array: probe function withstands a bending radius of 900 µm (2D model of rat cochlea). **(C)** insertion of a functional flexible µLED array into an isolated mouse cochlea through the round window at the base of the cochlea. The cochlear bone has been rendered semi-transparent by immersion in 2,2′-thiodiethanol (modified from [45••]).

targets the cochlea or the brain, flexible or stiff materials might be favored. The challenge with waveguides is their generally low coupling efficiency and additional light losses e.g. due to absorption. Active implantable solutions can build on important technological advances of optoelectronics regarding power-efficiency, downscaling and integration. Thin-film LEDs are available in various colors, have reached a power efficiency of more than 50% [44], can be miniaturized (µLEDs) and integrated into appropriate stiff or flexible substrates (e.g., [45••,46]). Moreover, collimation and focusing can be achieved via parabolic µLED surfaces [47] and combination with microlenses [48]. VCSELs though primarily available for infrared emission might serve spatially confined stimulation of neurons expressing red-shifted channelrhodopsins. Safe encapsulation and long-term stability will be required when developing these technologies for chronic animal experiments and future clinical translation. Regardless of choosing active or passive optical stimulators, a combination with electrical recording and, potentially, stimulation, will be of interest for both basic research and future clinical translation. Such 'hybrid devices' will be instrumental for comparative studies of optical and electrical stimulation and can serve as a back-up solution in future clinical optical CIs. Another major task is to devise control architecture and strategies for coding with multichannel optical implants. Developing auditory optical implants will benefit other optogenetic applications and vice versa (Figure 3).

Optogenetic stimulation of the auditory pathway is feasible and offers improved spectral resolution of coding

Given that neurons of the auditory pathway can exhibit substantial resting conductance [49,50] the potential for the low-conductance ChR2 [28•] for manipulating the firing in the auditory system has not been very clear. However, SGNs [51] and a number of principal neurons of the cochlear nucleus [49] are sufficiently 'tight' and, hence, seemed suitable for spike generation by ChR2. This has now been demonstrated by *in vivo* and *ex vivo* electrophysiological recordings of optogenetically driven activity [16••,31••]. Spiking of SGNs expressing ChR2 (transgenic mice, [33•]) or the improved ChR2-variant CatCh ([37•] via AAV-mediated transuterine gene transfer [36]) was triggered by blue laser light, which was delivered via an optical fiber inserted into the cochlea through the round window or a cochleostomy [16••,52•]. SGNs typically fired one spike (Figure 4a, b) and the

Figure 4



Feasibility and characteristics of optogenetic stimulation of the auditory pathway. (A) Responses of a single CatCh-expressing SGN to light stimulation: primarily a single action potential (arrow) was fired in response to 5 ms laser pulses delivered by an optical fiber inserted into the round window. Raising the stimulation rate increased the latency and jitter, but responses of the SGN were present at least up to 60 Hz (the maximum rate amenable to the set-up). The blue bar indicates the duration of light stimulation, eliciting stimulus artifats at on- and offset. **(B)** Reliability of spiking (proportion of trials eliciting a spike during the stimulation, top), mean first spike latencies (FSLs, bottom left), and FSL variance (bottom right) in response to 5- to 10-ms flashes at different intensities. Grayscale values indicate seven different light-responsive neurons in the region of the auditory nerve and cochlear nucleus. **(C)** Representative ICC current source density (CSD) patterns upon optical fiber (left), pure tone (middle), and monopolar electric (right) stimulation, respectively. Sinks are plotted in blue and sources in red. Significant sinks are outlined in black, with centroid and peak highlighted by black and white open circles. Note that multiple sinks were usually identified for electrical stimulation.

variance of spike timing (0.02 ms^2 [CatCh]-0.08 ms^2 [ChR2]) was lower than for stimulation with acoustic clicks (0.70 ms^2) [16••] and more similar to values reported for electrical stimulation [53]. CatCh-mediated spike generation followed optical stimulation up to at least 60 Hz (Figure 4a). CatCh-mediated first spike latency and variance decreased with increased intensity but increased with stimulus rate (Figure 4b). One major task for developing cochlear optogenetics is to increase the maximal firing rates, as upon acoustic and electric stimulation SGNs can follow stimulation at a few hundreds of Hz. The temporal fidelity of firing during suprathreshold optogenetic stimulation is primarily governed by the deactivation kinetics of ChR, although boosting K^+ channel-mediated hyperpolarization by enhanced Ca^{2+} permeation of the ChR can speed-up firing even despite a 20 ms deactivation [37•]. The most promising ChR for auditory optogenetics is the recently discovered variant Chronos, which has the shortest deactivation time constant described so far (approximately 3 ms at room temperature [54••]). In addition, Chronos — probably by improved expression levels — seems to overcome a conundrum, that is for a ChR to combine fast kinetics (photocycle) with high light sensitivity [29]. High light

sensitivity is another important demand for multichannel optical stimulation of the auditory pathway in basic research and future clinical application. Low energy consumption per pulse and channel will be critical to make the desired massive up-scaling of channel number compatible with battery lifetimes acceptable in behavioral experiments and clinical CI. The energy thresholds for optogenetic SGN stimulation was estimated in measurements of auditory brainstem responses (oABR), which indicated that the stimulus should exceed approximately 2 $\mu J/mm^2$ [16••]. This is lower than the estimated range for infrared stimulation (16–150 $\mu J/mm^2$, [19•]) but higher than the energy used per pulse in clinical CI (0.2 μJ, [22]). Clearly, measuring and lowering energy thresholds per pulse and channel for multichannel optogenetic stimulation is an important task for future work and might require further optimization of the ChR and its expression. Assessing the spectral resolution of cochlear optogenetics is only starting. Most informative so far was a comparison of the spatial spread of cochlear excitation between optical, acoustical and electrical stimulation obtained from measuring the spatial extent of activation in the auditory midbrain (inferior colliculus) [16••]. The central nucleus of the inferior colliculus (ICC) offers access to a well-ordered frequency representation (tonotopic map, e.g. [55,56]) that can be conveniently read-out by linear multielectrode arrays with each electrode reporting the activity of a given frequency band. A first characterization of the cochlear spread of excitation combined suprathreshold stimulation of basal SGNs (via a single optical fiber inserted into the scala tympani through the round window) with current source density analysis [57] based on local field potential recordings by multielectrode arrays (Figure 4c). As expected from the tonotopic organization, only the deepest ICC layers became activated, which were typically not acoustically sensitive due to the age-dependent high-frequency hearing loss in C57Bl/6 mice [58]. Electrical intracochlear stimulation, in contrast, caused of a multifocal activation of the ICC. Comparing the spatial extent of the largest current sink to that of the optically elicited ones revealed a significantly smaller cochlear spread of activation for optical stimulation, despite a quantitatively similar level of driven ICC activity [16••]. These experiments also provided evidence that optogenetic stimulation of the cochlea elicits activity in the central auditory pathway. Future work should explore the cochlear spread of excitation for lower light levels, multichannel optical implants and read out firing activity at the single-unit and multiunit levels at different stages of the auditory system.

Outlook

Following the initial demonstration of feasibility, much remains to be done to further establish, characterize and optimize optogenetic stimulation of the auditory pathway for basic research and clinical translation. Developing and characterizing rapidly gating, well expressing and potentially red-shifted ChR will help establishing optogenetics as a valuable stimulation option in auditory research and prosthetics. Postnatal transduction for ChR expression has been achieved in the central auditory system but needs to be established for the rodent cochlea most likely using AAVs followed by longitudinal studies of function and histology. Then, vectors and application technique need to be adapted to other species of interest, including nonhuman primates that will also serve to prepare and evaluate the approach in preclinical trials.

Developing optical multichannel stimulation for the cochlea and central auditory structures will require a multidisciplinary effort and should pursue 'active' and 'passive' lighting solutions. μLED arrays on flexible substrates, currently, seem to be the most advanced technology for optical cochlear implants, but important tasks such as appropriate encapsulation remain to be accomplished. Following tests of feasibility, employing optical multichannel stimulation requires the development of appropriate control architecture and coding strategies. Following characterization and optimization in acute experiments, longitudinal studies on chronically implanted animals will need to assess reliability and safety of the implants.

Finally, in depth characterization and optimization of optogenetic stimulation need to consider energy requirement, temporal bandwidth, frequency and intensity resolution of coding in comparison to acoustical and electrical stimulation. Importantly, beyond physiological recordings, this analysis needs to also to encompass behavioral testing for auditory percepts and characterizing the frequency and intensity resolution of coding. These studies will prepare the ground for the ground for innovative fundamental research on auditory function and dysfunction and likely pave the way for a groundbreaking advance of auditory prosthetics.

Conflict of interest statement

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