Toward the Optical Cochlear Implant

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When hearing fails, cochlear implants (CIs) provide open speech perception to most of the currently half a million CI users. CIs bypass the defective sensory organ and stimulate the auditory nerve electrically. The major bottleneck of current CIs is the poor coding of spectral information, which results from wide current spread from each electrode contact. As light can be more conveniently confined, optical stimulation of the auditory nerve presents a promising perspective for a fundamental advance of CIs. Moreover, given the improved frequency resolution of optical excitation and its versatility for arbitrary stimulation patterns the approach also bears potential for auditory research. Here, we review the current state of the art focusing on the emerging concept of optogenetic stimulation of the auditory pathway. Developing optogenetic stimulation for auditory research and future CIs requires efforts toward viral gene transfer to the neurons, design and characterization of appropriate optogenetic actuators, as well as engineering of multichannel optical implants.

In humans, disabling hearing loss has a major social, functional, and, with 360 million people worldwide affected, also strong economic impact (see who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss). Sensorineural hearing loss (SNHL) represents the most frequent type of hearing impairment. SNHL results from a dysfunction of the cochlea and/or the auditory nerve and the two most common causes are noise exposure (noise-induced hearing loss [NIHL]) and aging (age-related hearing loss [ARHL]), whereby NIHL and ARHL are mechanistically intertwined. In contrast to conductive hearing loss, which often can be alleviated by microsurgery of the middle ear in combination with active and passive mechanical prosthetics, as of today, no causal therapy for SNHL can yet

be provided in the clinic. Although hope has been generated by efforts in animal models to “repair” cochlear dysfunction in case of genetic hearing impairment by targeting genetic defects via viral gene replacement or genome editing (see Lustig and Akil 2018; Petit 2018), treatment of the more common NIHL and ARHL still seems further out of reach. Hence, hearing aids and cochlear implants (CIs) will remain the main options for hearing rehabilitation in SNHL for years to come and improving them is a major research objective. For the choice of instrument in a given SNHL case, the underlying reason is usually less important than its progression and its degree of severity. Generally, classic hearing aids are the choice for mild-to-severe hearing loss, while today electrical cochlear im-

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plants (eCIs) are available for profound hearing loss or deafness. However, when SNHL is primarily rooted in defective synaptic sound encoding as a result of impaired presynaptic (inner hair cells [IHCs]) or postsynaptic (spiral ganglion neurons [SGNs]) function (auditory synaptopathy/neuropathy; see Corfas 2018), hearing aids seem generally of little help and eCIs should be considered (Rance and Starr 2015; Moser and Starr 2016).

eCIs mediating electrical stimulation of SGNs via an array of 12–24 electrodes inserted into the scala tympani is considered the most successful neuroprosthesis with more than 500,000 registered users. Most of the eCI users achieve open speech understanding in the quiet, which is impressive, particularly given the shortcomings of eCI coding compared with physiology. In fact, the functional outcome highlights the power of neural computation used by the brain to relearn the processing of auditory information. What then are the limitations of current state-of-the-art eCIs? Most, if not all, colleagues would probably name the poor spectral resolution and the low dynamic range of sound coding (Shannon 2012; Zeng 2017). Both limitations result from the wide spread of current around electrode contacts (Kral et al. 1998) leading to cross talk between channels (Shannon 1983). They impede speech recognition in noisy environments and the recognition of music (Kohlberg et al. 2014). Physiologically, the perception of phonemes and speech in noisy environments is dependent on the resolution of intensity, represented by the dynamic range of the electrically driven SGNs (Zeng and Galvin 1999; Zeng et al. 2002), which is typically below 10 dB for eCIs (Zeng et al. 2008). Recognizing melodies and instruments relies on the spectral resolution of coding (Kang et al. 2009) and is also influenced by the channel cross talk. Therefore, the useful number of electrode contacts is typically limited to less than 10 (Friesen et al. 2001). Hence, research and technological developments toward better frequency and intensity resolution are necessary. Besides improvements of the speech processors, research currently aims at promoting neurite growth (Pinyon et al. 2014; Li et al. 2017) or inhibiting inflammatory responses

(Astolfi et al. 2016), at intraneural electrodes (Middlebrooks and Snyder 2008) or at multipolar stimulation configuration (Donaldson et al. 2005; Vellinga et al. 2017). Nonetheless, those approaches cannot counter the natural properties of electric current and its spread. This is why, recently, driven by advances in photonics, gene therapy, and optogenetics, the stimulation of SGNs with light has been proposed (Hernandez et al. 2014), promising a more spatially confined stimulation of the auditory pathway via multi-channel light-emitting-diode (LED) implants in the cochlea (Fig. 1) (for recent reviews, see Jeschke and Moser 2015; Moser 2015; Weiss et al. 2016; Richardson et al. 2017).

Figure 1. Comparing electrical and optical cochlear implants (oCIs). (A) Electrical cochlear implant (eCI), broad activation of neurons from each stimulation channel, channel cross talk, few channels. (B) oCIs, spatially confined activation, many channels.

The idea of improving the spectral resolution by means of optical stimulation is not entirely new. Stimulation of the cochlea with ultra-short and ultrastrong infrared light pulses was pioneered by Richter and colleagues (Izzo et al. 2007, 2008; Richter et al. 2011) building on the concept of direct infrared neural stimulation (Wells et al. 2007). The mode of SGN stimulation was suggested to be a direct optothermal effect altering membrane capacitance (Shapiro et al. 2012), but several studies have since then argued for an indirect optoacoustic phenomenon requiring hair cell transduction (Teudt et al. 2011; Schultz et al. 2012; Verma et al. 2014; Thompson et al. 2015). In contrast, optogenetic stimulation builds on a molecularly defined cell-specific mechanism and has dramatically lower energy requirements than stimulation by pulsed infrared light, regardless of the precise mechanism. In addition to their relevance for achieving reasonable battery lifetimes in future, optical cochlear implants (oCIs), introducing less energy and avoiding the absorption by water, are important for preventing excessive heating of the cochlea. In this review, we will focus entirely on optogenetic stimulation.

OPTOGENETICS—STIMULATION OF NEURONS WITH LIGHT

Since the characterization of the light-gated ion channels channelrhodopsins 1 and 2 (ChR1 and ChR2) in the green alga *Chlamydomonas reinhardtii* by Nagel and colleagues (2002, 2003), optogenetic applications gained a noticeable impact on research, especially in the neurosciences. In particular, the first successful transfection of mammalian neurons with ChR2 and the demonstration of reliable, fast, temporally precise, and noninvasive blue light stimulation to control action potentials of selected neurons showed the potential of optogenetics for controlling neural systems with high resolution (Boyden et al. 2005). In the following years, not only have many neural circuits been investigated with optogenetics, but also the technique itself has been refined, mainly by the discovery of further ChRs and ChR mutagenesis, yielding ChRs with different action spectra and kinetics allowing the

choice of channel to be adapted to the focus of the research (Yizhar et al. 2011). Moreover, in addition to manipulating the neuronal firing behavior (Adamantidis et al. 2015; Deisseroth 2015), optogenetics has arrived in cellular neurobiology as a means for controlling intracellular compartments (e.g., Rost et al. 2017).

Regarding the auditory system, the feasibility of ChR-mediated optogenetic neural activation has already been shown at the level of the cochlear SGNs (Hernandez et al. 2014), the cochlear nucleus (Shimano et al. 2013; Hight et al. 2015), the inferior colliculus (Guo et al. 2015), and the auditory cortex (Lima et al. 2009). Neural activity was read out at both the single-neuron and the neuronal population level along the auditory pathway. For example, optogenetic stimulation of SGNs caused activation of the auditory pathway up to the midbrain (Hernandez et al. 2014) and primary auditory cortex (Wrobel et al. 2018). This was used to provide first physiological evidence for a reduced cochlear spread of excitation with optical stimulation, which was further corroborated by mathematical modeling (Hernandez et al. 2014; Wrobel et al. 2018). Moreover, evidence for an auditory percept has been provided on optogenetic stimulation of SGNs (Wrobel et al. 2018) and the inferior colliculus (Guo et al. 2015). These experiments involved chronical optical implants and in case of Wrobel et al. (2018) also showed restoration of auditory percepts in deafened gerbils by oCI. In addition, efforts to optogenetically manipulate sensory cells have been undertaken (Wu et al. 2016), and optogenetics is currently combined with high pressure freezing to study the ultrastructure of functional states of hair cell synapses (C Wichmann, pers. comm.).

TUNING OPTOGENETICS FOR USE IN THE AUDITORY SYSTEM: OPSIN KINETICS, ION PERMEABILITY, ACTION SPECTRA, AND EXPRESSION

When developing optogenetics for use in future oCI, the choice of the optogenetic actuator needs to consider fast ChR kinetics for high-frequency stimulation and excellent expression conveying high light sensitivity. We consider a

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maximal SGN firing rate target of ~ 300 Hz for oCI. One of the most important features of ChRs that determines their activity characteristics is the opening and closing of the specific channel. Opening/closing of the first discovered ChR2 is based on a concerted movement of helices B, F, and G (Luecke 2001; Nakanishi et al. 2013) and the deactivation time constant (τ_{off}) is ~ 10 msec at room temperature (RT). The neural population response evoked by optical stimulation of the cochlea in mice expressing ChR2 under the control of the broad neural promoter Thy1.2 vanished at ~ 100 Hz (Hernandez et al. 2014), which seems low for encoding sounds. Some of this low temporal fidelity, however, might have resulted from prominent activation of nonauditory neural systems, as the amplitude of the responses (tens to hundreds of μV exceeded that of acoustic auditory brainstem responses [aABRs] up to ~ 10 μV in mice). Hence, a higher temporal fidelity of auditory neurons might have been overlooked. Indeed, with SGN-selective expression of the ChR2 variant CatCh ($\tau_{\text{off}} \sim 16$ msec) in the gerbil cochlea, optical ABR (oABR, which were slightly smaller than the aABR) remained sizable up to 200 Hz, and the stimulation rate-dependent reduction in oABR amplitude compared favorable to that of aABR (Wrobel et al. 2018).

Various point mutations close to the retinal binding pocket have been shown to affect the biophysical properties of the ChR, resulting in a variety of ChR-derived optogenetic tools. For example, the ChR2 mutant ChETA allowed sustained spike rates up to 200 Hz in fast spiking interneurons (Gunaydin et al. 2010). However, in our hands, ChETA-mediated optogenetic stimulation of SGNs was not successful, at least when using a transgenic mouse expressing ChETA (Keppeler et al. 2018). Recently, it was discovered that the interaction between the moving helix F and the virtually immobile helix C has major impact on ChR kinetics (Mager et al. 2018). Despite the lack of high-resolution structures of the most investigated ChRs, information on the high-resolution structure of the C1C2 ChR chimera in combination with the light-induced helix movement studies obtained by electron spin resonance (DEER) and the low-

resolution structure from 2D cryoelectron microscopy allowed identification of F219 in helix F as a crucial position for the construction of a faster ChR2 mutant. This residue is highly conserved in several other opsins, including the red-shifted opsins Chrimson (see below), ReaChR, and VChR1. Analogous mutations lead to accelerated kinetics of channel closing. Such mutants are of great interest for auditory optogenetics (Mager et al. 2018). In addition, screening of naturally occurring light-gated ion channels revealed the opsin Chronos (Klapoetke et al. 2014) as an interesting candidate for cochlear optogenetics.

Chronos seems to be the fastest naturally occurring ChR to date ($\tau_{\text{off}} \sim 3.6$ msec at RT) and has already been successfully used to drive auditory neurons in the cochlear nucleus and inferior colliculus (Guo et al. 2015; Hight et al. 2015). Our own recent work showed that Chronos closes with a time constant of less than a millisecond at physiological temperature (Keppeler et al. 2018). Membrane abundance of Chronos fused to enhanced green fluorescent protein (eGFP) when expressed in SGNs via adeno-associated virus (AAV6) under the human synapsin promoter was limited (see also Wrobel et al. 2018) and did usually not support functional stimulation of the auditory pathway. However, after adding sequences promoting exit from the endoplasmic reticulum (ES) (Ma et al. 2001; Stockklausner et al. 2001) and trafficking to the plasma membrane from the Golgi apparatus (TS) (Hofherr et al. 2005) as previously used in optogenetics (Gradinaru et al. 2010), and on using powerful AAV serotypes (AAV-PHP.B) (Deverman et al. 2016), the plasma membrane expression was enhanced and supported optogenetic stimulation (Keppeler et al. 2018). This Chronos-ES/TS version enabled optically evoked ABRs to follow stimulation up to 1000 Hz. Therefore, the temporal fidelity of Chronos-mediated optogenetic stimulation seems to meet the requirements of coding in the auditory nerve. However, and expectedly for the shorter channel open time, the energy requirement exceeds that found for ChR2 and the ChR2 variant CatCh, calling for further optimization of Chronos expression and membrane targeting.

Hence, Chronos remains the most promising candidate microbial opsin for blue cochlear optogenetics. However, we need to consider that chronic blue light stimulation of the cochlea might cause phototoxic damage to cells. Indeed, given the light power requirements, ChR2 or Chronos-mediated stimulation might exceed the regulatory limit set for the eye by the European Commission (see data.europa.eu/eli/dir/2006/25/2014-01-01).

Therefore, it is important to focus efforts toward generating fast, red-shifted ChRs. In addition to the phototoxicity aspect, red-shifted opsins have the advantage of reduced scattering and deeper penetration (Yizhar et al. 2011). Of the red-shifted opsins, Chrimson and its faster variant ChrimsonR (Klapoetke et al. 2014) have turned out to be very useful for various neural systems. However, neither Chrimson ($\tau_{\text{off}} \sim 21$ msec, RT) nor ChrimsonR ($\tau_{\text{off}} \sim 16$ msec, RT) seem fast enough for appropriate optogenetic control of the lower auditory pathway. Recent work has elucidated that faster closing kinetics of Chrimson can be achieved by replacing a tyrosine residue in position 261 of helix F by phenylalanine (Mager et al. 2018). Moreover, the Y261F mutation increased the relative calcium permeability (Mager et al. 2018) similar to that described for the ChR2 mutant CatCh (Kleinlogel et al. 2011). In addition to the Y261F mutation, two further mutations for Chrimson, S267M and Y268F, result in ultrafast switching behavior (Mager et al. 2018). The Y261F/S267M Chrimson double mutant (f-Chrimson, $\tau_{\text{off}} \sim 6$ msec at RT, 3 msec at physiological temperature [PT]) turned out to be expressed at high levels in the plasma membrane of cultured cells and in SGNs in vivo. The optically evoked ABRs extended to above 200 Hz and were quite comparable to acoustic stimulation. Analysis on the level of individual SGNs showed that spike probability and temporal precision (estimated as vector strength) declined for stimulation rates greater than 100 Hz. The very fast mutant Chrimson K176R/Y261F/S267M (vf-Chrimson), which additionally carries the K176R mutation (as described earlier) (Klapoetke et al. 2014 for ChrimsonR) shows an even shorter τ_{off} of 2.7 msec (RT, 1.6 msec at PT). Therefore, vf-Chrimson has ki-

netics comparable to Chronos (Klapoetke et al. 2014), until now the fastest ChR known.

In conclusion, ultrafast control of SGNs is available both for blue (Chronos) and red (vf-Chrimson) optogenetics. Given the shorter single-channel open time (lower open probability), the light requirements of these fast channels are substantial and need to be offset by optimized, plasma membrane expression. The advantages of red optogenetics make f- and vf-Chrimson excellent candidates for use in future oCIs.

VIRAL GENE TRANSFER INTO THE EAR—IMPLICATIONS FOR COCHLEAR OPTOGENETICS

Transgenic mice and rats expressing opsins in the nervous system, including the SGNs under the Thy1.2 promoter (Wang et al. 2007; Tomita et al. 2009), were useful for the first studies on cochlear optogenetics (Hernandez et al. 2014). However, for the method to be applied to animal models and finally to be translated into a medical application, other means to optogenetically manipulate SGNs are required. Delivery of genes with spatiotemporal control and cell-type specificity is one of the most important tasks for cochlear optogenetics, one that is shared with other efforts to achieve effective and safe gene therapy. Virus-mediated optogenetics offers flexibility for testing various ChR variants in a faster and more sophisticated way. Cell-type-specific manipulation typically builds on use of a particular promoter and the capsid of a specific viral subtype or spatial proximity to the source of application. To date, various vectors have been tested for cochlear gene delivery. Nonviral vectors, including plasmids alone administered via electroporation (Pinyon et al. 2014) or packaged within lipids or attached to nanoparticles (Praetorius et al. 2007), have the theoretical advantage of reduced toxicity and inflammation potentially associated with virally mediated gene transfer. However, they can be limited in transduction to immediately neighboring cells (Pinyon et al. 2014), might spread along the auditory pathway (Ge et al. 2007; Praetorius et al. 2007; Roy et al. 2010; Thaler et al. 2011; Zhang et al. 2011), and generally have low trans-

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duction efficiency and poor transgene expression patterns, limiting their usefulness (Staecker et al. 2001; Husseman and Raphael 2009). Major efforts have been undertaken in engineering and testing viral vectors as an alternative delivery method for various transgenes. The most promising candidates for cochlear gene delivery and future oCIs are discussed below.

Adeno-Associated Viruses

AAVs are naturally replication-deficient and require a helper virus for their replication. They are DNA viruses without any association to human diseases and are, hence, often considered non-pathogenic. Several AAV serotypes have proven successful for transduction of cells in the cochlea and auditory pathway. Thus, AAVs became a gold standard for efficient gene transfer into postmitotic hair cells and SGNs (Konishi et al. 2008; Reisinger et al. 2011; Akil et al. 2012; Hernandez et al. 2014; Jung et al. 2015; Emptoz et al. 2017). If not disabled, AAVs do integrate into the host genome at a specific and safe site. However, in most cases, AAVs are engineered not to integrate and form episomal DNA structures. Nonetheless, they permit long-term gene expression in postmitotic cells. Because of recent developments and improvements, AAVs are consequently used in optogenetics research and are arguably the best choice for cochlear gene therapy. Shu et al. (2016) provided valuable information about AAV serotypes and their potency for infecting different cell types in both early neonatal and adult rodent cochleae. To develop an atraumatic and noninvasive way of delivery, various AAVs were also tested for systemic application. Intravenous injection of rAAV2/9 carrying an eGFP reporter gene results in binaural transduction of IHCs, SGNs, and vestibular hair cells in a dose-dependent manner (Shibata et al. 2017). Moreover, transduction efficacy strongly correlated with the age of the animals with higher expression in neonatal mice.

An interesting approach to improve potency and cell specificity of AAV vectors applies Cre recombination-based AAV-targeted evolution (CREATE) in vivo. Using CREATE, Deverman et al. (2016) generated several novel AAV capsids

with higher prevalence to the central nervous system (CNS). One of them, called PHP.B, has more than a 40-fold higher efficiency than one of the standard AAV9 capsids after systemic application. Recently, introducing modifications of parental capsid version, even more specific versions for the peripheral nervous system (PHP.S) and more potent PHP.B (PHP.eB) for the CNS were generated (Chan et al. 2017). Via directed evolution, the potent capsid variant AAV2-7M8 was generated (Dalkara et al. 2013; Kotterman and Schaffer 2015) and proven to be an interesting candidate for delivering various transgenes to the rodent retina and CNS (Khabou et al. 2016) as well as primate retina (Hickey et al. 2017). Hence, directed viral evolution opens a door for future cochlear gene therapy and optogenetic applications. Taking a look back in a strategy called “ancestral reconstruction,” which is akin to an inversion of traditional molecular AAV evolution, two independent research laboratories significantly increased our knowledge of AAV capsid modifications (Santiago-Ortiz et al. 2015; Zinn et al. 2015). Zinn and colleagues identified Anc80L60 as a new predicted ancestor of the widely studied AAV serotypes 1, 2, 8, and 9. This highly potent AAV capsid was used in several recent publications showing its superior property as an in vivo gene therapy vector for targeting liver, muscle, and retina (Zinn et al. 2015). In 2017, Anc80 was successfully used to deliver the gene encoding eGFP to both neonatal and adult mammalian inner ear cells (Landegger et al. 2017a; Suzuki et al. 2017) and restored auditory and vestibular function in a mouse model of Usher syndrome type 1c (Pan et al. 2017).

In addition to potency and specificity, another very important parameter that determines the efficacy of AAV gene therapies is evasion from the host humoral immune response. Currently, the presence of neutralizing, preexisting anti-AAV antibodies can lead to the exclusion of patients from clinical trials. Therefore, the generation of AAVs that efficiently escape humoral and cellular immune responses is an important task. One of the strategies investigated to date is to associate AAVs with extracellular vesicles (“vexosomes”). Exosome-associated AAVs are currently in focus for gene therapy and

were successfully used for gene delivery into the murine retina (Wassmer et al. 2017) and into the murine inner ear to partially rescue hearing in a hereditary deafness model (György et al. 2017). With all these gene therapy tools, we are much closer to our final goal of having a suitable vehicle to deliver genes of interest and to be able to cure genetic dysfunctions or develop optogenetic approaches. AAVs have already been used successfully in clinical gene therapy trials (Dunbar et al. 2018), including those in the eye (Simonelli et al. 2010; Sahel and Roska 2013).

Adenoviruses

Adenoviruses are linear 36-kbp double-stranded DNA (dsDNA) viruses that infect a wide variety of organ systems, tissue types, and cells in humans without requiring cell division for transduction, making them good tools for transfecting the postmitotic, terminally differentiated cells of the mammalian inner ear. Adenovirus DNA remains episomal and does not integrate into the host genome, reducing the risk of insertional mutagenesis at the cost of mainly transient expression. Although initial studies showed substantial dose- and time-dependent hair cell toxicity (Dazert et al. 1997; Holt et al. 1999; Luebke et al. 2001a), multiply deleted ($E1^-$, $E3^-$, pol^- , pTP^-) adenoviruses without impaired viral infectivity (Holt 2002) preserved cochlear function and hair cell transduction (Luebke et al. 2001b; Holt 2002; Corey et al. 2004). However, because of their potential immunogenicity, cell toxicity, and possible CNS virus spread (Chen et al. 2015), future use of adenoviruses is under further investigation. Newer development of adenoviruses, mainly using helper-dependent adenoviruses (Wenzel et al. 2007) or different serotypes (Ad28) additionally supported their potential use in future cochlear gene therapy (Kraft et al. 2013). In fact, the first clinical trial aiming to drive hair-cell regeneration by transdifferentiation of supporting cells forced by the expression of Atoh-1 uses an adenovirus (ClinicalTrials.gov identifier: NCT02132130: Safety, tolerability and efficacy for CGF166 in patients with bilateral severe-to-profound hearing loss).

Lentiviruses

Lentiviruses belong to retroviruses that have RNA rather than DNA genomes. They are capable of infecting both dividing and postmitotic cells (e.g., neurons) and therefore widely used in neuroscience experiments. Because the DNA transcribed from viral RNA integrates into the host genome, lentiviral gene delivery leads to long-term expression. Targeting the cochlea already started in the late 1990s (Han et al. 1999), but lentiviruses transduce cochlear cells very poorly even in the presence of the transduction enhancer Polybrene (Han et al. 2015; Quan et al. 2015). However, lentiviruses could efficiently transduce rodent postnatal cochlear explants ex vivo (Maass et al. 2013). In conclusion, lentiviruses are potentially interesting as a tool to deliver transgene to cochlea, requiring further optimization and development.

Virus Application and Biosafety

Experience regarding biosafety of gene therapy approaches to the inner ear is still very limited. Generally, the biosafety of gene therapy vectors is dependent on four main interfering issues: (1) the vector itself, (2) its tropism, (3) the expressed protein, and (4) the application method.

1. As viral vectors developed for optogenetic approaches are typically AAVs, replication-incompetent, “nonpathogenic” viruses, their short-term biosafety is largely assumed, for example, from the modest immune response (Mingozzi and High 2013). Injection of AAV into the eye resulted in a temporary, if any, inflammatory response and did not seem to cause long-term immune responses (Simonelli et al. 2010). We speculate that just like in the eye, the ear, as an immune-privileged organ, might be less prone to develop an immune response. So far, insertional mutagenesis is considered to be the main risk for long-term safety (Russell 2007).
2. The tropism of the viral vector is dependent on the vector itself, but the choice of promoters seems to be more important for reliable, specific, and robust transduction as well as for light-sensitivity in optogenetics (Chaffiol et al. 2017). An SGN-specific promoter has

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yet to be determined to significantly increase biosafety via specificity of the optogenetic transfection to SGNs.

3. The expression of optogenetic proteins itself also has to be considered as potentially harmful. Although optogenetic tools have been used widely, few studies reported on apoptosis or on transformation of targeted cells. Anyway, those aspects definitely have to be considered, as, for example, chronic expression of ChR2 led to both in some experiments (Miyashita et al. 2013; Perny et al. 2016). Assessing safety concerns in vivo will be challenging, as they may be dependent on the protein, the properties of light stimulation, for example, wavelength and duration, as well as the tissue of interest. In our experiments on mice, we did not observe significant loss of SGNs at 9 months following early postnatal AAV6-mediated f-Chrimson expression (Mager et al. 2018).
4. The possibility of viral vector spread beyond the targeted area, for example, via endolymph-cerebrospinal fluid communication (Lalwani et al. 1996; Mager et al. 2018) and/or axonal transport (Sebastian et al. 2013; MacDougall et al. 2016), has to be considered for any application scenario.

Generally, we consider topical delivery to be superior to systemic delivery of the vector when it comes to targeting the inner ear in a (pre)-clinical setting for optogenetics. Although systemic delivery can be temptingly easy with intravenous or oral administration of the vector, topical delivery naturally decreases the risk of side effects and most probably also the spread of constructs. Available topical procedures to discuss include intralabyrinthine, trans-stapes, round-window, intramodiolar, and intratympanic application (Ogita et al. 2009; Staecker et al. 2014; Jung et al. 2015; Kurioka et al. 2016; Dai et al. 2017; Landegger et al. 2017b; Wrobel et al. 2018). Although transduction efficacy seems to decrease during development of the cochlea, a successful expression of CatCh in adult gerbils was recently obtained by direct intramodiolar injection of AAV6 (Wrobel et al. 2018).

TECHNOLOGICAL CONSIDERATIONS FOR THE DEVELOPMENT OF MULTICHANNEL OPTICAL COCHLEAR IMPLANTS

The characteristics of an oCI should be aligned to the current state of the art, the eCI. The major objectives for developing the oCI are to improve coding by enhancing spatial selectivity (frequency resolution) and dynamic range (intensity resolution). Feasibility of achieving better frequency and intensity resolution has been shown with waveguide-based stimulation of SGNs and computational modeling (Hernandez et al. 2014; Wrobel et al. 2018). However, further work is required to parametrize the cochlear spread of excitation, ideally, using realistic microscale emitters placed at various tonotopic positions along scala tympani. Ideally, the improvement in frequency and intensity resolution should not trade in the high-temporal fidelity of sound coding and low energy consumption of the eCI. Moreover, following decades of development cycles, current eCI technology features impressive reliability and longevity, which will be challenging to meet by replacing the electrode array with an emitter array that increases the number of stimulation channels by up to an order of magnitude, for example, in a case of an oCI with more than 100 micro-LEDs (μ LEDs).

Wafer-level processes enable the production of very dense arrays of “cell-sized” μ LEDs (Goßler et al. 2014) that can meet the requirements for the increased number of stimulation channels. Moreover, thin-film gallium nitride (GaN) LEDs achieve power efficiencies of >50% (Laubsch et al. 2010). However, replacing arrays of noble metal electrodes by “active” optoelectronics (Fig. 2) presents a substantial technological challenge regarding safe encapsulation and reliability. Moreover, as discussed above, blue light emitters such as GaN LEDs, dependent on the light power required, also pose a risk of phototoxicity and power-efficient microscale emitters in the green-orange spectrum are hard to make (“green-gap”). Finally, the question of heat generation and dissipation inside the cochlea requires attention. Should

Figure 2. Active micro-light-emitting-diode (μ LED)-based optical cochlear implant (oCI) versus passive waveguide-based oCI. (*Left*) Active μ LED-based oCI with μ LED array inserted into scala tympani. (*Right*) Passive oCI based on waveguide array, with optoelectronics encapsulated in the CI housing and waveguide array inserted into scala tympani. SP, Sound processor; C, cable; LS, light source; OF, optical fibers.

50% of the electrical energy fed in be converted to thermal energy, this might result in substantial heat generation dependent on the duty cycle of the emitters. Risk of potential leakage or heating is not expected when using waveguide-arrays as “passive” multichannel oCIs, piping the light from external emitters into the cochlea (Fig. 2).

With these considerations in mind, technological planning might consider the following tasks:

1. Implementation of prototypes, evaluation and decision making about active and passive oCI.
2. For the active oCI this will need to involve:
 - a. the assessment of different types of microscale emitters such as LEDs, laser diodes, and vertical cavity surface emitting lasers (VCSELs),
 - b. further development of orange-red microscale emitters for low phototoxicity stimulation,
 - c. further efforts to enhance the power efficiency and, for the case of LEDs, beam-shaping, and

- d. development of safe and long-term stable encapsulation, yet sufficient flexibility for atraumatic cochlear insertion.
3. For passive oCI this will require:
 - a. safe enclosure of optoelectronics and efficient optical coupling,
 - b. sufficient transmission, low losses, directed projection, and
 - c. stable optical properties of the emitters and waveguides.
 4. Implementation of new coding strategies considering the available number of stimulation channels.

In conclusion, we expect that this technological development will require major efforts by academia and industry. In our view, the success of this work will be just as important for using cochlear optogenetics as implementing efficient expression of the opsin in SGNs.

COMPARING OPTOGENETIC AND ELECTRICAL STIMULATION

Enhancing the spatial selectivity of stimulation (spread of excitation) and consequently increasing the frequency and intensity resolution is a major aim of the oCI. A first study indicated a smaller spread of cochlear excitation for supra-threshold optical single-fiber versus monopolar electrical stimulation by current source density analysis in the inferior colliculus (Hernandez et al. 2014). Moreover, the output dynamic range estimated based on optically evoked ABRs (≥ 20 dB) (Hernandez et al. 2014; Mager et al. 2018; Wrobel et al. 2018) exceeded that reported for eCI (typically ≤ 10 dB) (Zeng et al. 2008). Nonetheless, clearly more work is required to quantify the frequency and intensity resolution gained over the eCI. The spatial selectivity is not only dependent on the activator, but also governed by the site of excitation in SGNs. In acoustic hearing, SGN spiking is driven by excitatory postsynaptic currents into the postsynaptic bouton and thought to be generated at the first heminode in the organ of Corti near the synapse (Rutherford et al. 2012), which

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warrants the best frequency resolution possible. In the case of electrical stimulation, excitation might occur at the axon initial segment and the nodes of Ranvier of the SGN (Tehovnik et al. 2006), additionally limiting the spatial selectivity alongside to the current spread from the activating electrode, as axons of originally distant SGNs passing by the stimulation site could be activated. The site of optogenetic stimulation would depend on how the projection of light is implemented: toward Rosenthal's canal housing the SGN somata or toward the peripheral neurites. The choice of projection will depend on the integrity of the SGNs, that is, the availability of intact peripheral neurites. Future efforts to characterize the dependence of the spread of excitation on the direction of projection should include computational modeling (Hernandez et al. 2014), measurements of spread of excitation in the inferior colliculus, as well as studies that involve ChR-targeting to specific cellular compartments, that is, soma versus neurites.

Electrical stimulation in CIs is a tough benchmark for cochlear optogenetics regarding the temporal precision and energy requirement of coding per pulse. However, we argue that not all features of eCI coding should be implemented for oCIs. For example, physiological firing rates for SGNs in response to continued strong depolarization amount to 200–300 Hz (Lieberman 1978; Winter et al. 1990; Taberner and Liberman 2005; Strenzke et al. 2009). However, current eCIs stimulate at rates even above 1000 Hz per contact, introducing stochasticity into the neural activation because of partial refractoriness, thus avoiding a nonphysiological synchronization of SGNs (Rubinstein et al. 1999). For optical stimulation, the desired stimulation frequency is under discussion. oCIs may not require such pseudostochasticity as smaller groups of neurons are addressed and natural variability of excitation arises from different expression levels of ChRs among the SGNs and their variable proximity to the emitter. We expect that preclinical studies will help identify the ChR of choice for balancing the requirement of temporal fidelity and energy consumption. However, comparison of the first study that estimated an energy threshold of 2 μ J per pulse for

optical ABRs (Hernandez et al. 2014) elicited by ChR2-mediated stimulation ($\tau_{\text{off}} \sim 10$ msec, RT), and a study that found 0.5 μ J per pulse for f-Chrimson ($\tau_{\text{off}} \sim 6$ msec, RT) (Mager et al. 2018) suggests that high expression levels might offset the “disadvantage” of shorter open times. Still, the energy per pulse used for supra-threshold stimulation in eCI is 4 to 10 times lower (0.2 μ J) (Zierhofer et al. 1995). This, together with scaling up the number of stimulation channels, indicates a greater power requirement of the oCI, which can likely be offset partially by reducing the pulse rate per stimulation channel.

ROADMAP AND PROPOSED WORKFLOW

Developing the oCI for use in auditory research and for future clinical translation requires major complementary efforts in (1) optogenetic manipulation of the SGNs, (2) technological development of optical stimulators and appropriate control hard- and software, and (3) physiological and psychophysical characterization in animal models, using the eCI as a benchmark. Should these preclinical studies indicate the necessary feasibility and safety (1/2), as well as major advantages in sound coding over eCI (3), even greater efforts will be required to transfer and approve the oCI technology to human use. The possible workflow to apply oCIs in humans will include the same steps needed for implantation of eCIs with additional application of the vector for the light-gated ion channel. Whether single- or two-step procedures for implantation of the oCI and application of the vector will be reasonable cannot be determined so far as it will strongly depend on the final vector and its preferred application. In the end, the potential superiority of optical to electrical stimulation has to be proven, considering frequency and intensity resolution of stimulation and energy consumption. To replace the widely used and successful eCI, we will not only have to show the advantages of optical stimulation for, for example, the appreciation of music, but also to focus on developing a long-lasting, reliable optical implant and a safe gene transfer procedure.

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