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Challenges for the application of optical stimulation in the cochlea for the study and treatment of hearing loss

Rachael T. Richardson[a,b], Alexander C. Thompson[c], Andrew K. Wise[a,b] and Karina Needham[d]

[a] Bionics Institute, East Melbourne, Australia;

[b] Department of Medical Bionics, University of Melbourne, East Melbourne, Australia;

[c] School of Physics and Astronomy, University of St Andrews, St Andrews, UK;

[d] Department of Surgery (Otolaryngology), University of Melbourne, Royal Victorian Eye & Ear Hospital, East Melbourne, Australia

Abstract

Introduction: Electrical stimulation has long been the most effective strategy for evoking neural activity from bionic devices and has been used with great success in the cochlear implant to allow deaf people to hear speech and sound. Despite its success, the spread of electrical current stimulates a broad region of neural tissue meaning that contemporary devices have limited precision. Optical stimulation as an alternative has attracted much recent interest for its capacity to provide highly focused stimuli, and therefore, potentially improved sensory perception. Given its specificity of activation optical stimulation may also provide a useful tool in the study of fundamental neuroanatomy and neurophysiological processes.

Areas covered: This review examines the advances in optical stimulation – infrared, nanoparticle-enhanced, and optogenetic-based – and its application in the inner ear for the restoration of auditory function following hearing loss.

Expert opinion: Initial outcomes suggest that optogenetic-based approaches hold the greatest potential and viability amongst optical techniques for application in the cochlea. The future success of this approach will be governed by advances in the targeted delivery of opsins to auditory neurons, improvements in channel kinetics, development of optical arrays, and innovation of opsins that activate within the optimal near-infrared therapeutic window.

1. Hearing loss and neural prostheses

Hearing loss is a major health and economic burden on society, affecting an estimated 360 million people worldwide [1]. Hearing loss can result in significant communication disorders with educational, social and vocational ramifications that adversely affect quality of life, and incur substantial cost to the health system and loss of productivity.

Cochlear hair cells are sensory cells that transduce the acoustic input to the inner ear by eliciting nerve action potentials in the auditory neurons (ANs) that form synaptic connections with them. However, the cochlear hair cells and their neural connections are highly susceptible to damage (Figure 1A-B) which is commonly caused by exposure to loud noises, disease or ageing. In severe cases there is widespread hair cell loss meaning that the inner ear is no longer responsive to acoustic input. In these cases the only therapeutic intervention is a cochlear implant, a neural prosthesis designed to electrically stimulate residual ANs to provide the spectral and temporal cues necessary for speech perception. Cochlear implants enable recipients to understand speech in good listening conditions, but do not work well with competing background noise and do not enable the appreciation of music or similar complex sounds [2].

Cochlear implants take advantage of the tonotopic organisation of the cochlea (that is also preserved throughout the auditory pathway) by processing the acoustic signal into discrete frequency bands that are mapped to specific electrodes along the array. A significant amount of information is also carried in the temporal structure of acoustic signals [3] which the implant uses to independently modulate the intensity of stimulation in each frequency band. In principle, electrical stimulation of a single electrode site with a temporally modulated signal will excite a well-defined cochlear region. In practice, however, neural excitation is spatially very broad due to the conductive nature of cochlear fluids resulting in poor spatial precision [4-6] (Figure 1C). As a consequence, the stimulating channels overlap considerably so that any single neuron can be activated by multiple electrodes. Implant recipients therefore perceive low resolution spectral information. Interactions between stimulating channels also result in the distortion of the temporal signal significantly reducing sound quality and speech perception [7, 8]. Finally, because channel interaction caused by simultaneous currents often results in uncontrolled loudness of perception, electrical

stimuli are typically delivered sequentially to a single electrode at a time [9, 10]. This minimizes channel interactions but results in the loss of the fine temporal structure of the acoustic signal.

It is widely anticipated that more localized intracochlear stimulation (i.e. improved precision) would result in an increase in the number of independent stimulating channels (i.e. separate AN subpopulations) available for activation, thereby improving sound perception for cochlear implant recipients. This cannot be achieved by simply increasing the density of electrodes along the electrode array due to the highly conductive cochlear fluids. Optical stimulation is not limited by the same conductive 'spread' and therefore has the potential to improve the precision of neural activation from a cochlear implant (Figure 1D).

2. Optical stimulation as a novel form of neural stimulation

Focused, pulsed light (nanosecond-millisecond duration) offers an alternative means of neural stimulation to overcome the problem of current spread. As the principal benefit of this approach includes non-contact stimulation with high spatial resolution, it promises to overcome many of the limitations of electrical stimulation, namely poor spatial specificity via broad current spread. Given the aforementioned constraints of neural activation via the cochlear implant, optical stimulation has been proposed as a novel solution. Infrared neural stimulation and near-infrared stimulation have been explored as methods for direct stimulation of ANs, while nanoparticle-assisted and optogenetic–based stimulation methods require prior manipulation of ANs before they can be applied. These will be discussed in turn.

2.1 Infrared stimulation in the cochlea

Infrared laser light activates neurons by transient and localized heating of water within cells that absorb the infrared light (infrared neural stimulation; INS). The potential of INS was first demonstrated in the rat sciatic nerve [11] using a free electron laser and diode laser (λ = 2120 nm). Since this initial demonstration, INS has been applied to a number of different neural targets, including the facial nerve [12], auditory nerve [13], visual cortex [14] and embryonic heart [15]. Typically, radiant exposures of 300 – 1000 $mJ.cm^{-2}$ have been used, with pulse repetition rates restricted to 10 Hz in order to avoid thermal damage [16, 17].

The use of INS to activate ANs in the cochlea has been tested in a range of animal models, including gerbil [13], guinea pig [18] and cat [19]. The first trial of INS in the cochlea (*in vivo*) reported compound action potentials (CAPs) in response to stimulus [13]. Significant effort has since been placed on exploring the INS parameter space in the cochlea [20, 21]. These efforts have revealed that, unlike other neural targets, cochlear INS requires lower radiant exposures (typically 5 – 100 $mJ.cm^{-2}$), thereby allowing repetition rates up to 250 Hz without evidence of thermal damage during acute experiments [16, 19]. It is unclear how the increasing heating from higher repetition rates of up to 900 Hz used in contemporary cochlear implants will be tolerated. To date, no experimental work has explored higher stimulation rates, whilst modelling of stimulation rates up to 1000 Hz suggests heating of up to $10^{o}C$ [16]. Recent work has also explored the potential of near-infrared light (NIR; λ = 800 - 1000 nm), as this has lower absorption in water and consequently deeper penetration through tissue [22-24].

Although a range of mechanisms are understood to drive neural activity as a result of light-induced heating of tissue, the situation in the cochlea is complicated by the presence of a photoacoustic or optoacoustic effect. That is, an acoustic artefact (pressure wave) resulting from rapid heating of water [12]. Thus where INS is applied in animals with remaining hair cells, the functional hair cells still present in the cochlea likely detect the pressure wave [25-29] in addition to, or instead of a direct activation of the neurons. To eliminate residual hearing as a cause of neural activity, two deafened models have been employed. In the first model, in which neomycin was added and allowed to diffuse through the cochlea, neural responses to INS were maintained following a 30-40 dB impairment in acoustic thresholds [18], but this shift in hearing threshold is unlikely to represent complete elimination of all hair cell function. Other studies have been unable to generate a response in the profoundly deaf cochlea (> 50 dB impairment), where hair cells are rendered non-functional following perfusion of neomycin from the round window to the oval window [28, 30]. Adding to the controversy, conclusions from acoustic masking in normal hearing and hearing impaired animals [31] suggest that acoustic artefact is not the dominant mode of neural activation. This is supported by evidence that the magnitude of INS responses appear highly dependent on positioning the radiation

beam path toward the AN population [32]. Nonetheless, others have shown that the pressure waves generated by infrared lasers are sufficient in amplitude to stimulate the partially deafened cochlea [33].

Concurrently, the molecular mechanisms by which neural activity can be evoked by infrared light have been examined across many *in vitro* models, and a number of possible mechanisms have been proposed. Examining INS (1875 nm) in retinal and vestibular neurons, Albert, Bec and colleagues [34, 35] reported laser-evoked neuronal voltage variations in both cell types, and demonstrated activation of action potentials, which were confirmed by blocking with the sodium channel specific tetrodotoxin. These events were also blocked by ruthenium red and RN 1734, suggesting thermal activation of the vanilloid transient receptor potential (TRPV) ion channel family. This outcome supported earlier *in vivo* outcomes in the cochlea that indicated TRPV1 channels could contribute to the neural response to optical stimulation [36]. However, subsequent electrophysiological studies in cultured ANs (the neural population targeted by cochlear implant stimulation), found laser (1450 nm and 1870 nm) stimulation induced changes in membrane voltage that were incapable of producing action potentials [37-39]. These findings instead appeared to support the idea of a reversible change in membrane capacitance as first proposed by examination of 1869-1889 nm stimulation in model cells (oocytes and HEK cells) and artificial lipid bilayers [40]. Nonetheless, modeling data [41] suggested that capacitance is unlikely to be the only mechanism at play. An alternative possibility is a triggered increase in intracellular calcium [42-44]. This mode of activation has been demonstrated via calcium imaging in both ANs and vestibular neurons, in which infrared light (1863 nm) induced calcium transients that were found to be attributable to mitochondrial sources, and not membrane-bound sodium, potassium or calcium permeable channels [43].
Together these findings suggest that whilst INS can work in some specific *in vivo* scenarios and cell models, there is little *in vitro* or *in vivo* evidence that auditory neurons are reliably and sufficiently stimulated by infrared light to make INS a viable technique in the deaf cochlea.

2.2 Nanoparticle-assisted neural stimulation

A complementary approach to INS is the use of an external absorber such as gold nanoparticles that render the neurons more sensitive to light-induced heating [45, 46]. Amongst the external absorbers, gold nanoparticles provide an ideal candidate for biological applications as a non-immunogenic material. Through control of their size, aspect ratio and surface dielectric properties, gold nanoparticles can be tailored to absorb light within a very narrow near-infrared range. A significant advantage of this approach is that red to near-infrared light (750-950 nm) can penetrate deeper within tissue, requiring lower energy than INS alone. When irradiated the gold nanoparticles can produce rapid heating due to the photon-to-heat energy conversion, and induce intracellular calcium transients [45, 46]. Application of this method to cochlear ANs demonstrated success in stimulation of neural activity *in vitro* [47]. Using silica-coated gold nanorods tailored to absorb light at 780 nm, action potentials and inward currents were evoked in ANs [47]. Alternatively, no response could be evoked from ANs incubated with silica-coated gold nanospheres (with absorption maxima at 525 nm) or ANs cultured without nanoparticles during 780 nm illumination [47].

Nonetheless, given the infancy of research in this field there remain a number of challenges to address in efforts to safely implement nanoparticle enhanced INS. These concern nanoparticle delivery, neural targeting, biocompatibility and longevity. As with the delivery of any exogenous product or drug, it is imperative to ensure that the delivery of nanoparticles is contained within the cochlea, and the delivery method does not introduce or increase infections that might put the remaining cell populations at further risk of degeneration. Once delivered, the task is to ensure that nanoparticles can localize within or upon their target cell population. The recent findings of Carvalho-de-Souza [48] show good promise in this regard. Gold nanoparticles conjugated to ion channel ligands successfully targeted dorsal root ganglion neurons, which responded to infrared stimulation with action potentials. This nicely demonstrates a complementary method of targeting specific cell populations, which would be an important consideration in any future application of this technique *in vivo*. This approach may be most applicable in the periphery, where neural populations are present in discreet ganglia. Yet with the highly conserved nature of biology, the challenge in targeting to the central nervous system will be in finding a membrane bound protein that is

unique to the target population alone. This also raises the question of the stability or longevity of membrane bound nanoparticles. Studies examining the long-term retention of nanoparticles in the cell will be required. A related hurdle is toxicity. Our current knowledge around short-term internalization of nanoparticles appears to suggest that it is safe to the extent that neural activity is maintained, and repetitive stimulation is not damaging [47, 48] in the way INS alone is [37]. However, the effects of large temperature gradients on cell viability over the longer-term are yet to be established, casting some doubt over the suitability of optical stimulation techniques that rely on internal temperature shifts.

3. Optogenetic tools

The relative lack of efficacy of infrared stimulation of ANs has prompted investigation of optogenetics as an alternative approach. The recent emergence of optogenetic techniques has enabled auditory neurons to be stimulated by relatively low powered light sources, providing a potential strategy to overcome the limited precision of contemporary bionic devices as stated above. Optogenetics is a term that refers to the use of genetic techniques to introduce light sensitive channels (opsins) into the plasma membrane of specific neuronal populations. Opsins function by depolarizing or hyperpolarizing the neuron via ion fluxes in response to pulses of light [25, 49-51], enabling the activation or silencing of neurons with unprecedented spatial and temporal precision. The benefit of introducing opsins into neurons is that responses can be elicited at an energy threshold that is around 8 to 70 times lower than those reported for infrared stimulation and could potentially be reduced to a level only twice as high as electrical stimulation [52]. By introducing light sensitive ion channels, this approach also bypasses the need to induce activity via temperature shifts.

Opsins can be either excitatory, for instance Channelrhodopsin-2 [53], or inhibitory, for example halorhodopsin (NpHR)[54]. Channelrhodopsin-2 is activated by blue light (440-500 nm) while the chloride channel based halorhodopsin is activated by yellow light (560-590 nm). Many variants of opsins have been discovered or engineered to change their activation wavelength, time scale of activation or sensitivity to light stimulus [49]. For example, a calcium translocating variant of channelrhodopsin-2, termed CatCh, has

accelerated response times to the light stimulus and is 70 times more sensitive to light than channelrhodopsin-2 [55]. The increased light sensitivity and fast kinetics make CatCh and similar channelrhodopsin-2 variants suitable candidates for biomedical applications.

3.1 Optogenetic neural stimulation

Optogenetic tools were first trialled in the auditory system in 2009. The distinct optical response of channelrhodopsin-2-expressing neurons in the auditory cortex was used to tag and identify specific neuronal populations during electrophysiological recordings [56]. A few years later, Shimano and colleagues [57] assessed the effect of expression of channelrhodopsin and halorhodopsin on the activity of neurons in the dorsal cochlear nucleus. That study found no deleterious effects of expression of the opsins on the response of the neurons to acoustic stimulation and showed that optical stimulation with blue light could elicit responses in the cochlear nucleus. Furthermore, channelrhodopsin-2 was found to be localized in the soma and dendrites of ANs, thereby highlighting its potential as a useful tool for tracing neuronal projections [57].

The first reported use of opsins in the peripheral auditory system (the cochlea) to enable optical activation of the central auditory pathway was in 2014 [58]. Overexpression of channelrhodopsin-2 in cochlear ANs was achieved via the use of a previously established transgenic mouse line [59] in which channelrhodopsin-2 expression is mediated by the Thy1 promoter with strong expression in ANs. This study also investigated expression of a channelrhodopsin variant with improved kinetics (CatCh), first described by Kleinlogel in 2011 [55]mediated by transuterine AAV gene transfer [58]. An optical fiber was then inserted into the cochlea via a cochleostomy or through the round window membrane. A blue laser was pulsed in the cochlea while electrophysiological recordings of optically-driven activity were made in central auditory structures. As the intensity of the light was increased, the reliability of spike generation increased and latency decreased, as occurs with electrical stimulation. In order for an auditory brainstem response to be recorded, 2 $\mu J.mm^{-2}$ of energy was required to stimulate ANs, far lower than for INS, but still 10 times the amount used per pulse in cochlear implants. In further comparison to electrical stimulation from cochlear

implants, optical stimulation of even the fast CatCh variant permitted only low rate stimulation up to 60 Hz, an order of magnitude lower than contemporary cochlear implants using electrical stimulation.

The idea behind optical stimulation of ANs is to reduce the spread of activation that is encountered with electrical stimulation. The maintenance of tonotopicity in the inferior colliculus means that recordings from this nucleus can be used to measure the spread of auditory nerve activity [4, 60]. Thus in the Hernandez paper [58], spread of activation in the cochlea was evaluated by recording local field potentials in the central nucleus of the inferior colliculus in channelrhodopsin-2 transgenic mice in response to acoustic, electrical or optical stimulation. These local field potentials were then transformed into current source densities. The authors measured the spatial extent of the major current sinks along the tonotopic axis of the inferior colliculus and found that the spread of activation for optical stimulation was similar to acoustic stimulation and significantly less than for electrical stimulation suggesting more restricted activation of ANs [58].

With the rapid pace of development of opsins, faster kinetic opsins are likely to be developed that incorporate high light sensitivity and the fast channel kinetics that would be required for optogenetic neural stimulation of the auditory system. Indeed, Chronos is a variant of channelrhodopsin with both faster on/off firing rates and higher photosensitivity [61]. The temporal resolution of channelrhodopsin-2 and Chronos were directly compared following AAV-mediated expression in the dorsal and ventral cochlear nucleus. Blue light optical stimulation was applied to the cochlear nucleus while neural activity was recorded upstream in the inferior colliculus which receives direct projections from the cochlear nucleus. The application of the study was discussed in context of auditory brainstem implants (ABI), an alternative option to cochlear implants that are typically provided to patients in which cochlear implants would be ineffective, for example, people with structural defects of the cochlea or damage to the central auditory nerve as a result of injury or surgical intervention. In these situations a cochlear implant is not effective as any neural signal elicited within the cochlea is not reliable transferred to higher auditory brain structures as a consequence of central nerve damage. ABIs are implanted onto the surface of the cochlear nucleus to

provide direct stimulation of the cochlear nucleus, typically using rates of 250 pulses per second. As with other devices, ABIs would benefit from more localized stimulation for better comprehension of speech as well as reduced side effects [62]. In terms of channel kinetics, Chronos was better able to maintain synchrony of firing with the optical stimulus at a rate of up to 224 pulses per second compared to channelrhodopsin-2 [63], suggesting that it may overcome some of the limitations of the slow channel kinetics of channelrhodopsin-2.

The success of optogenetics is contingent on the ability to transduce neurons with opsins. The first report of optogenetic stimulation in the cochlea used embryonic gene transfer methods to express opsins in auditory neurons [58]. However, to be clinically viable, opsins must be introduced into adult ANs specifically, permanently, efficiently and extensively throughout the cochlear spiral without affecting residual hearing. Gene therapy will be the most likely tool that can transfer opsin transgenes into ANs and satisfy all of the above criteria. Viral vectors can be delivered locally to the cochlear fluids using an approach that is similar to the surgical technique used for insertion of a cochlear implant in humans [64]. The following sections will discuss the use of gene therapy as it relates to optical stimulation of the auditory system.

3.1 Preservation of residual hearing

Preservation of residual hearing is highly desirable in order to allow for combined electrical and acoustic stimulation of the auditory system for improved pitch, speech and music perception [65]. The same is likely to apply for optical stimulation. Unfortunately, some loss of residual hearing can occur with direct injection of viral vectors into cochlear fluids due to i) the opening of the cochlea, and ii) loss of cochlear fluids during injection [66-68]. This is not unique to gene therapy and is a common side-effect of cochlear implantation in patients with residual hearing. As far as injecting viral vectors into the cochlea is concerned, potential loss of hearing can be ameliorated by improving the injection technique such as injection or diffusion of viral vectors through the round window membrane [66, 69-71], making this a likely clinical route for introduction of transgenes such as opsins into the cochlea. The effect of expression of channelrhodopsin on

AN physiology and hearing was not reported by Hernandez et al [58], but gene therapy *per se* does not have a deleterious affect AN on survival or function, and expression of channelrhodopsin or halorhodopsin in the auditory brainstem of adult rats does not affect hearing [57].

3.2 Duration of channelrhodopsin expression

Permanent expression of opsins in ANs will be required for life-long optical stimulation. Viral vectors, particularly adeno-associated virus (AAV), provide a means for long-term or possibly even permanent transgene expression in neuronal cells. AAV-mediated expression of channelrhodopsin (CatCh variant) in AN membranes of mice persisted for 2 months while AAV-mediated channelrhodopsin and halorhodopsin expression in the dorsal cochlear nucleus of adult rats persisted for 18 months, both of which were the longest time points tested [57, 58]. Channelrhodopsin has been stably expressed in retinal neurons for up to 12 months in mice via AAV gene transfer [72, 73], but long-term gene expression in ANs has not been tested. Other gene therapy studies in the cochlea predict that AAV-mediated gene expression is likely to persist long-term, with AAV1-mediated restoration of hair cell function for 9 months in mice (the length of the study)[74], adenovirus-mediated transgene expression in guinea pig cochleae for at least 6 months [75] and stable AAV-induced transgene expression in the brain of a non-human primate over 8 years [76]. Channelrhodopsin-2 has also been safely expressed in the brain of non-human (rhesus macaque) via lentivirus gene transfer [72]. All of these studies suggest long-term opsin expression in human ANs is achievable.

3.3 Efficiency of transduction

Gene therapy in the cochlea has been extensively studied, but some of the challenges to overcome include efficiency and specificity of transduction. The rate of transduction in the cochlea is highly dependent on age, with rates of transduction decreasing in older animals [74]. While VGLUT gene therapy in newborn mice resulted in 100% transduction of inner hair cells, this reduced dramatically to 40% by two weeks of age [74]. Even with the same conditions, transduction can vary with resulting variation in the expression of opsins, as was found for expression of channelrhodopsin-2 in the cochlear nucleus following direct viral

injection, where two-thirds of the injected mice were positive for channelrhodopsin-2 [77]. Nevertheless, transduction of cells in adult cochleae is possible, with neurotrophin gene therapy significantly protecting ANs from degeneration after hearing loss [75]. In another study [58], it was found that 40% of ANs were transduced following embryonic AAV-mediated delivery of CatCh and that this was sufficient to elicit an optogenetic response in the auditory midbrain. However, it remains to be determined how many ANs need to be transduced to obtain benefits of optical stimulation over electrical stimulation and how variability in expression affects the response.

3.4 Extent of transduction

In many gene therapy studies, the area of transduction is largely limited to the injection site. Transduction efficiency decreases with distance from the injection site [68, 75, 78]. The success of optogenetics for optical stimulation in the cochlea will require efficient transduction throughout the cochlea, or at least along the length of the optical array. One study used the cochlear implant itself to not only deliver the genetic material (naked complementary DNA) to the cochlea, but also to deliver the electrical stimulus required to transfect cells in the vicinity of the implant via electroporation [79]. While this technique was applied to brain derived neurotrophic factor and did not transfect ANs, the premise could be applied to viral vectors that can penetrate further into tissue and have greater specificity for the AN population.

3.5 Cell specificity

AAV gene transfer technology is likely to be the first choice for expressing channelrhodopsin variants in ANs. One of the many advantages of AAV is the availability of different serotypes that display different cell specificities. Some serotypes of AAV have more specificity for ANs than others. In a direct comparison of different AAV serotypes it was found that AAV serotype 5 resulted in the highest transduction of ANs in the cochlea, but also serotypes 1 and 2 to a lesser extent [71]. In another study, transduction of ANs was achieved with bovine AAV [80]; an AAV serologically distinct from primate serotypes that would allow for gene therapy in a patient with neutralizing antibodies to other AAVs [81]. AAV technology allows for directed modifications that improve transduction and efficiency. In the visual system, efficient uptake of

AAV by retinal ON-bipolar cells (the target neuronal population) required targeted mutagenesis of the AAV8 capsid to improve penetration into the tissue, binding and transduction. CatCh, a fast variant of channelrhodopsin-2, was then successfully expressed in retinal ganglion cells using a specific promoter with subsequent successful optogenetic stimulation of the visual system [82].

4. Conclusion

Cochlear implants are devices that electrically stimulate ANs to return some function to people with severe to profound hearing loss. The resolution of stimulation by cochlear implants is limited by current spread, which results in broad neural excitation and channel interaction. More localized stimulation could be achieved with optical stimulation, as light can be focused. We have presented a review of work on optical stimulation, covering infrared stimulation, near infrared stimulation as well as stimulation using the visible spectrum of light, although the latter requires ANs to be modified with a light-responsive ion channel in order to initiate an action potential. It has been demonstrated that channelrhodspin-2 and its variants can be used for optical stimulation of ANs in the cochlea, with initial reports that optical stimulation reduces the spread of activation and may therefore help to achieve more localized stimulation. Optical stimulation therefore has the potential to allow more independent stimulating channels for improved perception of speech and music.

5. Expert opinion

Optical neural stimulation techniques are emerging as an exciting alternative to conventional electrical current-based forms of neural activation that are used in neurophysiology and bionic devices. The key advantages are i) improved spatial resolution, and ii) ability to target specific neural populations. The driving force behind application of these techniques to the cochlea is the expected improvement in perception outcomes through the development of next generation cochlear implants. Optical stimulation may also provide a unique opportunity to improve our understanding of auditory physiology by permitting targeted stimulation of specific neuronal populations within the auditory pathway, and therefore a method of interrogating a component of the auditory network in isolation.

The properties of light in tissue and water vary greatly over the different wavelengths of light used for optical stimulation and play a key role in determining the suitability of different optical stimulation techniques. When targeting distinct neural populations, light propagation in tissue is limited by the two photophysical properties: absorption and scattering. Absorption limits the depth that light can penetrate into tissue, while scattering limits both the depth and the spatial confinement of light (Figure 2). These properties depend on the wavelength of light and the media or tissue the light is propagating through. Typically, shorter wavelengths have significantly higher scattering and tissue absorption, while longer wavelengths have higher water absorption (Figure 3). The wavelength range of 800 – 1000 nm balances these competing limitations and is considered to offer an ideal "therapeutic window" for optical treatment. Nanoparticle-assisted and near-infrared neural stimulation are within this range, while current optogenetic-assisted stimulation (visible spectrum) and INS have limited tissue penetration due to higher scattering and absorption. Nevertheless, developments in this field are moving toward the development of opsins that activate within such a therapeutic window [83], enabling use of more optimal wavelength in the future. For applications in the auditory system, where i) distances between implants and target neurons are relatively short (<500 μm) and ii) the bone separating the ANs from the scala tympani (where the electrode resides) is very thin (6-25 μm)[84], the theoretical limitations of blue light optogenetic stimulation already present a significant improvement over conventional electrical stimulation, a result supported by preliminary experimental studies [58]. It is worth noting that light scattering is unlikely to affect spatial resolution as scattered light will not have enough energy to activate neighboring AN populations.

Application of INS, nanoparticle-assisted INS and optogenetics to the cochlea has provided useful insight to the potential and limitations that are specific to the auditory system. Infrared and near-infrared neural stimulation have a distinct advantage in not requiring any modification to target neural tissue, however it has significantly higher energy requirements than optogenetic- and nanoparticle-enhanced INS. For INS the energy requirement is typically 1 to 2 orders of magnitude higher than optogenetics, while near-infrared neural stimulation can be up to 4 orders of magnitude greater [24]. This results in significant heating of the

cochlea, typically raising the temperature by 1 – 2 °C [16, 19]. While chronic implantation studies have found no deleterious effects from stimulation at a single site [85], the increased thermal load from a multi-channel array and higher pulse repetition rates currently has an unknown risk to neural and supporting tissue. In the cochlea, the controversy surrounding the mechanisms of INS from acoustic artefacts must be resolved before the clinical potential of this technique can be evaluated. This is a key challenge as the target patient group for cochlear implants typically lack the hair cells that may mediate the response attributed to INS. Further, in those patients with some remaining functional hair cells, the photoacoustic effect would consistently activate a specific subset of hair cells and their respective ANs, in addition to the real target neurons therefore misrepresenting the frequency characteristics of the acoustic waveform.

Optogenetics can ensure that light will evoke neural activity whereas INS appears to be somewhat reliant on channel composition of the target neural population, and can also be damaging to tissue. The success of optogenetics in the future will depend on targeted delivery of opsins to the ANs. Virus-mediated transfer of opsins to ANs is a promising approach for experimental and future clinical translation of optogenetic-assisted optical stimulation of the cochlea. A clinical trial is underway that will help test the clinical safety and efficacy of viral-assisted gene transfer to the cochlea. The GenVec Inc/Novartis trial is testing the efficacy of adenoviral-mediated expression of a transcription factor in the cochlea of profoundly deaf people for hearing restoration [86]. While the transgene is very different to opsins, the trial will help determine the overall safety of viral-mediated gene therapy in the cochlea. The vast majority of gene therapy clinical trials involve the use of AAV as a gene transfer tool. AAV-mediated transduction of ANs with opsins is likely to be persistent (based on numerous AAV gene therapy studies) and safe, with current evidence suggesting that opsins do not affect the survival or function of neurons.

It is currently very difficult to transduce ANs throughout the entire cochlear spiral using viral gene transfer techniques. Injection of viral vectors into the basal region of the cochlea typically results in transduction of cells that are near the injection site, with some spread to the middle turns of the cochlea. This is likely to be sufficient for optical stimulation of the cochlea as the optical array, much like the current cochlear implant,

will not reach the apical turns of the cochlea and stimulation of apical ANs will not be possible. Using embryonic gene transfer methods, approximately 40% transduction of ANs can be achieved [58]. Efficiency of transduction will need to be improved to near 100% of the AN population in order to make the most of the tissue available for optical stimulation. Additionally, inter-subject reproducibility of transduction will need to be improved. Both efficiency and reproducibility improvements are likely to come from research into injection methods and devices [87, 88]. Given the evidence of stable, long-term gene expression achieved with AAV and its low risk of integration or delayed adverse events, AAV appears to be the most suitable gene transfer technology for optogenetic stimulation of ANs. AAV has been approved for use in numerous clinical trials to date and is considered low risk within Food and Drug Administrations (FDA) guidelines for cellular and gene therapy.

Cochlear implants currently have stimulation rates that range from 250 pulses per second to 2,400 pulses per second. The deactivation speed of channelrhodopsins limits the rates at which optical stimulation can be applied to the channelrhodopsin-expressing neurons. While variants are still being manufactured and discovered, the current fast-rate variants still fall behind electrical stimulation rates with synchronization of optical stimulation and firing, failing to match up to the standard set by electrical stimulation. The use of a channelrhodopsin variant that can stimulate ANs at close to 250 pulses per second, such as Chronos, would be an ideal starting point. Energy thresholds for optical stimulation are still higher than cochlear implants but it is likely that thresholds can be reduced through improvements in channelrhodopsin expression levels and through the discovery of variants with higher light sensitivities.

Delivering light to many individual locations in the cochlea also presents a significant engineering challenge. There are two broad approaches for this challenge: using waveguides to direct light to stimulation sites, or positioning miniature light sources at the point of stimulation. Techniques to integrate optical fibers into cochlea electrode arrays have been developed both with the intention of developing an optical array [89, 90] and for measuring array insertion forces [91]. The results from Balster and colleagues [89] are the most promising, with the successful insertion of an array containing 8 optical fibers into a human cochlea without

causing damage. The advantage of this approach is that heat generating LEDs or laser diodes are kept away from the cochlea. However, losses from coupling into the miniature fiber may be too large to make it a viable technique. The field of optogenetics has spurred development of miniature light sources [92] and many of these techniques can be applied to the cochlea. Highly flexible cochlear arrays developed around μLEDs have been developed with up to 10 individual channels [93, 94] and present a viable solution to an optical array. Present devices cause moderate tissue heating (0.5 – 1˚C) [94, 95], however further improvement to device and opsin efficiency may reduce this.

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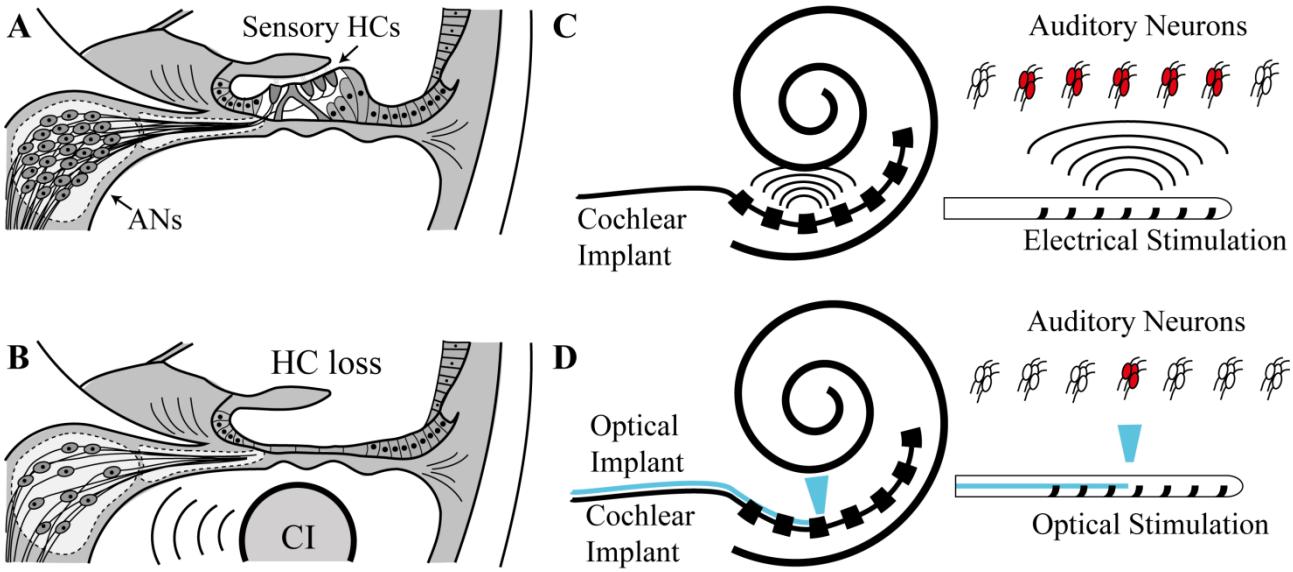

Figure 1. **(A)** Schematic of a normal cochlea depicting the sensory hair cells (HCs) and auditory neurons (ANs). **(B)** In a deaf individual loss of sensory HCs and supporting cells leads to significant loss of the ANs. A cochlear implant (CI) can be used to electrically activate residual ANs to return some auditory function. **(C)** Electrical stimulation activates a broad population of neurons due to current spread. **(D)** Optical stimulation in comparison will activate a more restricted region of the neural population.

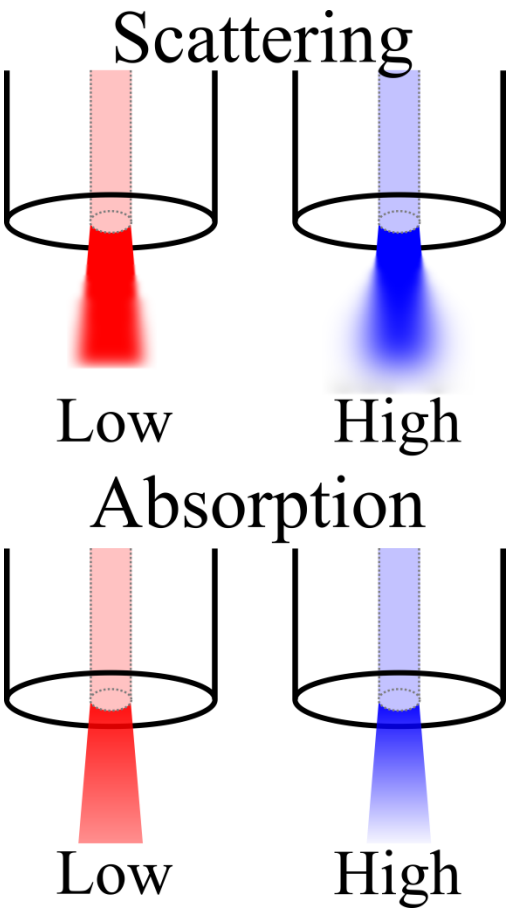

Figure 2. Illustration of the behaviour of light in low and high scattering or absorbing media. In the scattering case, blue light typically exhibits stronger scattering in biological tissue and has reduced penetration in tissue. For absorption, a more strongly absorbing media, light intensity reduces at a smaller depth compared to a media with less absorption.

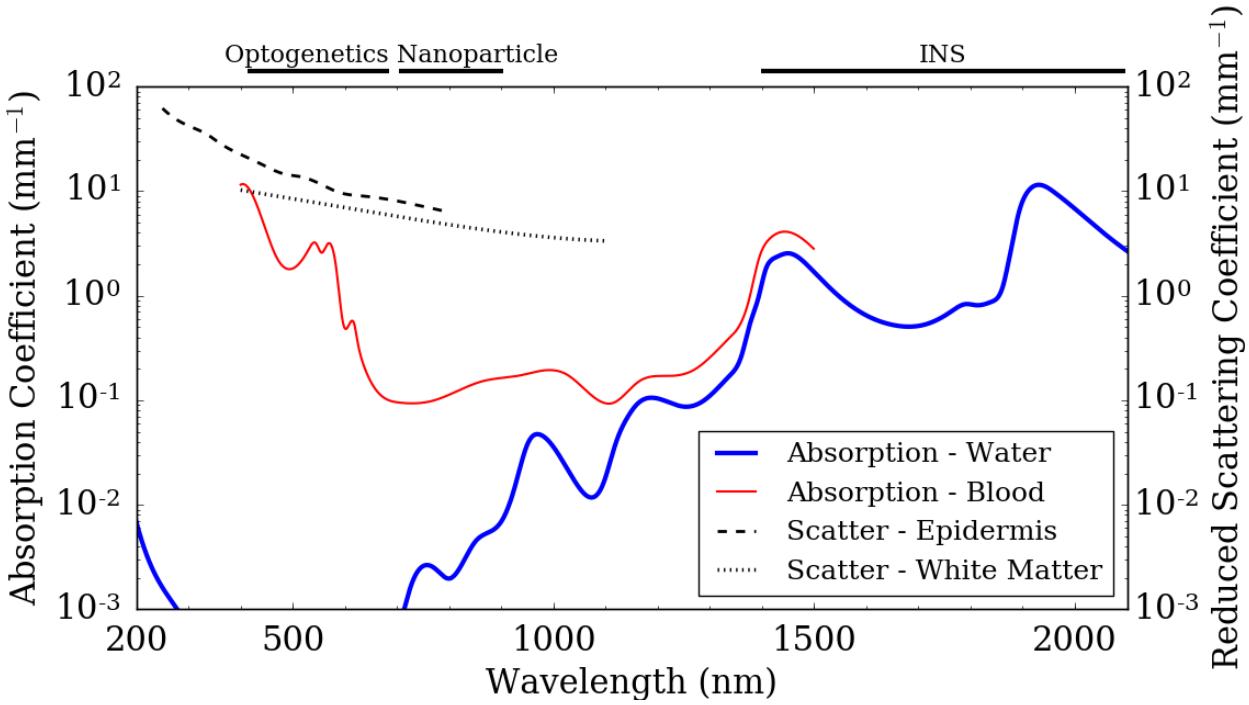

Figure 3. Illustration of optical properties in tissue over the wavelength ranges used for optical stimulation. Highlighted sections show those commonly used for optogenetics (380 – 650 nm), nanoparticle-enhanced INS (700 – 900 nm), and INS (1400 – 2200 nm). Absorption coefficient is shown for water [96] and oxygenated blood (5% haematocrit)[97] and the reduced scattering coefficient spend for epidermis[98] and white matter [99, 100].