

# Optical Stimulation of Neurons

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**Abstract:** Our capacity to interface with the nervous system remains overwhelmingly reliant on electrical stimulation devices, such as electrode arrays and cuff electrodes that can stimulate both central and peripheral nervous systems. However, electrical stimulation has to deal with multiple challenges, including selectivity, spatial resolution, mechanical stability, implant-induced injury and the subsequent inflammatory response. Optical stimulation techniques may avoid some of these challenges by providing more selective stimulation, higher spatial resolution and reduced invasiveness of the device, while also avoiding the electrical artefacts that complicate recordings of electrically stimulated neuronal activity. This review explores the current status of optical stimulation techniques, including optogenetic methods, photoactive molecule approaches and infrared neural stimulation, together with emerging techniques such as hybrid optical-electrical stimulation, nanoparticle enhanced stimulation and optoelectric methods. Infrared neural stimulation is particularly emphasised, due to the potential for direct activation of neural tissue by infrared light, as opposed to techniques that rely on the introduction of exogenous light responsive materials. However, infrared neural stimulation remains imperfectly understood, and techniques for accurately delivering light are still under development. While the various techniques reviewed here confirm the overall feasibility of optical stimulation, a number of challenges remain to be overcome before they can deliver their full potential.

**Keywords:** Infrared neural stimulation, neural engineering, neural stimulation, optical stimulation, optogenetics.

## 1. INTRODUCTION

Despite the success of electrical stimulation for triggering neurons, there has been interest in improving the efficacy of electrical stimulation or developing alternative techniques without the disadvantages and limitation of electrical stimulation [1, 2]. The usefulness of many bionic implants has been limited by the spread of current from the electrodes [3] and the lack of spatial specificity [2]. Similarly, neuroscience would benefit from the ability to turn individual neurons on or off [1]. There has long been interest in using light to influence the behaviour of neurons [4-6] partially as it is less invasive than other techniques.

Light has been known to influence the behaviour of neurons since the work of d'Aarsonval in 1891 [4]. While there are some neurons, such as photoreceptors in the eye, that have become specialised in order to respond to light, most neurons are not normally activated by exposure to light [7]. Fork [5] showed that abdominal ganglion neurons in *Aplysia californica* respond to 488 nm laser light, through a reversible mechanism, despite the cells not being photosensitive. Building on the work of [5] and others, Balaban *et al.* [8] found that He-Ne laser irradiation ( $\lambda = 632.8$  nm) of subesophageal ganglia of *Helix pomatia*

promoted membrane depolarisation and action potentials in spontaneously active neurons. Hirase *et al.* [6] demonstrated that neurons could be triggered through exposure to pulses of light from a femtosecond laser via a two-photon mechanism. More thorough reviews of these early developments can be found in the references [2, 9] and readers are referred to these.

Over the past decade, a number of new techniques have been developed to use light to trigger neurons. Early techniques were able to increase the sensitivity of neurons and generate action potential spikes with laser exposure timescales of seconds [10, 11]. These techniques include genetically modifying the target neurons to introduce photosensitive receptors in the neurons [10, 12], or the introduction of photoswitchable molecules that bind to specific ion channels and modify their behaviour when exposed to light [11, 13, 14]. In contrast, advancements in laser diode technology for infrared neural stimulation has allowed the use of wavelengths that are directly absorbed by water [2, 15]. This paper reviews the various optical techniques of nerve stimulation currently under development, with a particular focus on infrared neural stimulation due to its minimally invasive characteristics.

## 2 OPTOGENETICS AND PHOTOACTIVE MOLECULES

### 2.1. Optogenetics

Optogenetics is a technique to introduce light-sensitive ion channels to neurons, allowing them to be switched on or

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off optically with tight spatial and temporal confinement [16, 17]. Although early attempts at introducing light-sensitive channels had allowed tight spatial localisation of stimulation, temporal responses were on the order of seconds [10, 18]. Despite the temporal limitation, these strategies were able to induce behavioural changes in a *Drosophila* model [19].

The expression of Channelrhodopsin-2 (ChR2), a rapidly-gated blue (470 nm) light-sensitive cation channel, in oocytes of *Xenopus laevis* and mammalian cells introduced a technique to depolarise cells in timescales on the order of milliseconds [20]. Application of this to neurons was developed by Boyden *et al.* [12]. In that work, neurons were transfected with ChR2, introducing light sensitivity and allowing driving of single spikes with light. Introduction of photosensitivity to neurons *in vivo* was soon demonstrated [21], showing the potential of optogenetics for both *in vitro* and *in vivo* studies. These advances significantly boosted the field by allowing precise temporal control of neuronal firing exposed to blue light.

Since the development of ChR2 as a light-activated channel, a range of new opsins have been developed to allow inhibition of neurons, faster stimulation rates and response at longer wavelengths [22-24]. Halorhodopsins respond to light near 580 nm and act as a chloride pump, inhibiting excitation of neurons by hyperpolarisation [23, 24]. Other work has found that high stimulation rates with ChR2 can have an inhibitory effect [25], potentially allowing the one opsin to both excite and inhibit neural activity. Genes to express opsins are typically delivered with viral transfection [12], but electroporation transfection [26] and optical transfection [27] have also been demonstrated as a feasible techniques. While ChR2 gave a large improvement in response times compared to previously used opsins, it has a typical maximum response rate of 40 Hz [22] and there has been interest in developing faster responses. As there is interest in applying optogenetic techniques to neuroprosthetic implants such as the cochlear implant [28-31], higher stimulation rates may be needed to match the electrical stimulation rates that are commonly used. Recent developments have allowed spike trains of up to 200 Hz with novel opsins [22], although further development will be needed to reach the 900 Hz stimulation rate used in the cochlear implant [32]. Another limitation of ChR2 is the high scattering of 470 nm light used for excitation [33]. Much research has been focused on developing opsins with longer maximum excitation wavelengths where scattering and absorption in tissue is lower [9]. Advances include a cation channelrhodopsin (VChR1) responding at 589 nm [34] and ReaChR an opsin responding at 610 nm [35]. While promising, these opsins do not offer as fast switching as blue excitatory opsins [9].

Optogenetics have been utilised to further our understanding of neural disorders [36], understand neural systems and encoding [37] and there is interest in using optogenetics to treat blindness and Parkinson's disease [17, 38, 39]. Despite the power of these tools and their importance in the field of neuroscience, the potential for use in bionic devices or treatment of conditions in humans is currently limited.

Optogenetic techniques require transfection of genes into neuronal cells, which, besides technical challenges, will face significant regulatory hurdles for human use [40, 41].

## 2.2. Photoactive Molecules

Another technique to optically activate neurons relies on neurotransmitters that are held in a photosensitive cage but can be liberated upon exposure to light [17]. Caged glutamate is one of the most commonly used molecules for stimulation [42], but a number of neurotransmitters have been used [43]. The short wavelengths that were initially used to photoactivate the cages ( $\lambda \sim 380$  nm) tended to limit the spatial resolution of this technique, due to the high level of light scattering in tissue [44] and severely limit probing depth due to strong absorption. Furthermore, the high intensity of light and high energy of photons required for uncaging can damage tissue. The development of two-photon responsive glutamate cages allowed both finer spatial resolution and deeper penetration into tissue [45], as uncaging can occur with exposure to 800 nm light. Further refinement has reduced the optical radiation required by improving efficiency of the two-photon uncaging process [44] or developing cages which release upon exposure to visible light [46, 47]. Caged molecules are also able to inhibit neural activity, through the release of GABA [46, 44]. However, this technique requires further development as many of the compounds used also interact with the receptor before photoactivation [44].

Another approach to control neurons with photosensitive molecules is the use of bistable molecules to create photoswitches. These molecules have multiple configurations, which can change upon exposure to light of different wavelengths [17, 18, 48]. This property has given rise to photoswitchable ligands, which can regulate  $K^+$  channels and glutamate receptors [17, 49]. There is considerable potential to extend this approach by engineering new molecules for modulating a wider range of specific targets.

In addition to control of neurons, techniques are emerging to read neural activity with light [50]. These techniques include sensing of cell membrane potential, calcium and neurotransmitter release [50]. Complete optical control and monitoring of neural activity could allow the study of larger and more complex neural systems than is allowed by current electrophysiology techniques.

Caged molecules have emerged as a key technique for determining functional connectivity of neurons [44, 51]. It has been used to map the functional synaptic connections in the visual cortex [51], the cerebral cortex [52], and many other areas [53]. Despite the success of caged molecules as a tool for fundamental neuroscience studies, they have limited potential for *in vivo* studies or for neuroprosthetic implants due to the need to replenish the caged neurotransmitter and potential toxicity of the cage [17].

## 3. INFRARED NEURAL STIMULATION

Infrared light has been demonstrated as an alternative technique for optical stimulation of nerves without the need

for genetic manipulation or other interventions [2, 15]. The technique of using infrared light to stimulate neurons has been coined infrared neural stimulation (INS). The use of infrared light has a number of potential advantages over electrical stimulation: finer spatial resolution can in principle be achieved, no direct contact between the stimulation source and target neurons is required, there is no electrochemical junction between the source and target tissue, and there is no stimulation artefact on the recording electrodes. Disadvantages of INS include: heating of the tissue to level that could cause damage [54, 55] and a restriction on the maximum depth of stimulation due to absorption of light in the intervening tissue [55]. Compared to optogenetic and caged molecule techniques, INS requires no modification of the target tissue as it only relies upon the absorption of infrared light by water in the tissue [2, 56].

Pulses of mid-infrared light were first observed to elicit responses in mammalian nerves by Wells *et al.* [15]. They exposed the sciatic nerve of rats to irradiation from a free electron laser (FEL), with wavelengths between 2000 nm and 10,000 nm, observing compound nerve action potentials (CNAP) and compound muscle action potentials (CMAP) with a strong spatial specificity. Additionally a Ho:YAG laser ( $\lambda = 2120$  nm) produced a response in the sciatic nerve. Histological analysis of the nerves after stimulation showed no evidence of tissue damage, confirming that the energy deposited is below tissue damage thresholds. This work was

expanded upon in [57], where the dependence of the damage and stimulation thresholds on wavelength, and therefore absorption coefficient, were further investigated. Wavelengths with lower water absorption ( $\mu_a \sim 3 \text{ mm}^{-1}$ ), were found to have a greater safety ratio between the energy required for stimulation and the threshold for damage.

Since the initial demonstration of INS in the rat sciatic nerve by Wells *et al.* [15], the technique has been extended and demonstrated in a number of other models. A summary of these other targets is presented in Table 1, along with the laser wavelength, pulse length, fibre diameter and resultant threshold found. It is not intended as a comprehensive summary of all INS studies, but rather to provide an overview of the main targets that have been investigated.

### 3.1. INS in Peripheral Nerves

Work in the rat sciatic nerve has been extended to examine both the safe range of stimulation parameters [58] and a comparison between optical and electrical stimulation modalities [59]. When optical and electrical stimulation modalities were compared, Wells *et al.* [59] found a near linear relationship between the radiant exposure and the measured compound nerve action potential (CNAP) response, similar to that observed when using electrical stimulation. Unlike the electrical response, the minimum CNAP response from an optical stimulus was four times

**Table 1.** Summary of the various experimental parameters for a range of different INS studies in the literature. This table does not present data from every study discussed in this section, but provides an overview of the main results.

Author	Neural Target	Wavelength (nm)	Stimulation Threshold (mJ.cm <sup>-2</sup> )	Pulse Length (ms)	Fibre Diameter ( $\mu\text{m}$ )
Wells [15]	Rat sciatic nerve	2120, 2000 – 6100 <sup>1</sup>	320	0.25	600
Wells [58]	Rat sciatic nerve	2120	340	0.35	600
Wells [59]	Rat sciatic nerve	2120	320	0.35	600
Teudt [60]	Gerbil facial nerve	2120	710	0.25	600
Fried [61]	Rat cavernous nerve	1870	1000	2.5	300
Fried [62]	Rat cavernous nerve	1850 – 1880	350	2.5	400
Jenkins [63]	Embryonic quail heart	1875	810	2	400
Jenkins [64]	Adult rabbit heart	1851	7000	2.5 – 12	400
Cayce [65]	Rat somatosensory cortex	1875	140 <sup>2</sup>	0.25	400
Izzo [66]	Gerbil cochlea	2120	18	0.25	100
Izzo [67]	Gerbil cochlea	1844 – 1873	6	0.035 – 1	200
Izzo [68]	Gerbil cochlea	1923 – 1937	1.6	0.05 – 0.3	200
Richter [69]	Gerbil cochlea	1844 – 1873	3 <sup>3</sup>	0.03 – 1.6	200
Duke [70]	Rat sciatic nerve	1875	1690 <sup>4</sup>	2	400
Duke [71]	Aplysia buccal nerve	1875	8930	2 – 3	100

<sup>1</sup>At discrete wavelengths of 2100, 2500, 3000, 3500, 4000, 4500, 5000, 5500 and 6100 nm.

<sup>2</sup>Threshold not well defined, a change to intrinsic signal was observed at 0.14J.cm<sup>-2</sup>.

<sup>3</sup>Chronically deafened animals displayed greatly increased thresholds (over 10-fold), especially at longer pulse lengths.

<sup>4</sup>Increased threshold compared to work by Wells *et al.* is due to smaller core diameter. Threshold reduced three-fold upon application of simultaneous electrical pulse at 95% threshold.

smaller. This lower minimum response suggests greater spatial localisation with optical stimulation. Stimulation of the rabbit sciatic nerve with 1875 nm light has also shown the strong spatial localisation of INS [72]. The authors found that not all of the nerves' surface was sensitive to INS and that maximum INS levels typically recruited 2–9% of any muscle. The reduction in recruitment may be due to the increased size of the rabbit nerves compared to previous studies with rats.

The suitability of other wavelengths of infrared light for stimulating the rat sciatic nerve was performed by McCaughey *et al.* [73]. In this work wavelengths of 1540 nm, 1495 nm, 1540 nm and the more conventional 2100 nm were examined, using various diode laser sources. Stimulation was achieved with all laser sources, however it was most reliable when using the 1495 nm source. However, meaningful comparisons between the different wavelengths and laser sources is made difficult by the large variation between fibre diameters, beam divergences and pulse durations. Moreover, the long pulses ( $\geq 100$  ms) used by the 1450 nm and 1540 nm sources are near the thermal diffusion time for the areas irradiated [74].

The gerbil facial nerve was stimulated using INS by Teudt *et al.* [60]. The authors used a Ho:YAG laser ( $\lambda = 2120$  nm,  $\phi_{\text{core}} = 600$   $\mu\text{m}$ ,  $t_{\text{pulse}} = 250$   $\mu\text{s}$ ,  $f = 2$  Hz). Response to the irradiation was observed when using radiant exposures from 0.71 J.cm<sup>-2</sup> to 1.77 J.cm<sup>-2</sup>, with amplitudes similar to that observed when using electrical stimulation. Histological analysis of higher radiant exposures revealed that damage was present at levels greater than 2.00 J.cm<sup>-2</sup>. In addition to measuring the response of nerves, the authors measured the profile of the beam resulting from transmission in air, Ringer's lactate and muscle tissue. No change in the beam profile was observed when transmitted through Ringer's lactate when compared to air. However, transmission through tissue was found to broaden the spot when compared to the other media. These results showed that Beer's law is a good first approximation for the spatial behaviour of light during INS, but scattering also plays a role in the propagation of light. The results suggested that INS could be beneficial clinically as a monitor of the facial nerve during surgery, as no contact to the nerve is required and it allows for greater spatial selectivity.

The rat cavernous nerve was stimulated using INS by Fried *et al.* [61] to establish the potential of INS for nerve mapping during nerve-sparing radical prostatectomy. A thulium fibre laser ( $\lambda = 1870$  nm,  $\phi_{\text{core}} = 300$   $\mu\text{m}$ ,  $t_{\text{pulse}} = 2.5$  ms,  $f = 10$  Hz, radiant exposure = 1.00 J.cm<sup>-2</sup>) was used. The intracavernosal pressure was monitored and showed a similar response to the laser stimulation as occurred with conventional electrical stimulation. This result demonstrated the feasibility of INS for noncontact stimulation of the cavernous nerves. The technique was further optimised in [62], where a tuneable laser (1850 nm  $> \lambda > 1880$  nm,  $\phi_{\text{core}} = 400$   $\mu\text{m}$ , rep rate = 10 Hz) was used. Optimal parameters of 1860 nm  $< \lambda < 1870$  nm with a minimum

radiant exposure of 0.35 J.cm<sup>-2</sup> were found. Further work [75] investigated the use of continuous wave lasers ( $\lambda = 1455, 1490, 1550$  nm) rather than the pulsed laser system previously used [62]. A wavelength of  $\lambda = 1490$  nm with corresponding water absorption of  $\mu_a = 1.9$  mm<sup>-1</sup> was found to provide the best performance, balancing absorption with penetration depth. The continuous wave system was found to give a faster intracavernosal pressure response compared to the pulsed laser system. The authors conclude that while promising, translating the technique from the rat model to human patients may be difficult as the target nerves are harder to visually identify in humans.

The heart is another target of INS that has seen interest [63, 64, 76]. Jenkins *et al.* [63] demonstrated that the heart of an embryonic quail could be paced using INS as a non-invasive technique. 1875 nm laser light was used and no damage was observed in the tissue. Optical pacing of the heart was further explored in [64]. Here, pulses of 1851 nm light were used to pace adult rabbit hearts at radiant exposures of 6–11.8 J.cm<sup>-2</sup> and pulse duration of 2.5 ms to 12 ms. Unlike other results for INS, a pulse duration of 8 ms was found to have the lowest radiant exposure stimulation threshold. Additionally, higher stimulation frequencies resulted in a lowering of optical thresholds, similar to that observed by Duke *et al.* [77]. Using propidium iodine staining to determine damage, radiant exposures of 7.9 J.cm<sup>-2</sup> or above per pulse were found to cause some disruption of cell membranes, which may limit the duration over which this technique can be used.

An attempt to stimulate nerves *ex vivo* was made by Cargill *et al.* [78]. They used a diode laser ( $\lambda = 1850$  nm,  $P_{\text{peak}} = 5$  W,  $\tau_{\text{pulse}} = 1-5$  ms,  $\phi_{\text{core}} = 600$   $\mu\text{m}$ ). Ten nerve samples were extracted from mice and responded to electrical stimulation. However, no activation was observed when using optical stimulation. The authors speculated that the explanation for this discrepancy between *ex vivo* and *in vivo* may be due to the nerves being at room temperature rather than body temperature, or due to other differences between the mouse and rat animal models.

### 3.2. INS in the Central Nervous System

Due to the improved spatial localisation of INS, it has attracted interest as a technique to stimulate the central nervous system [65, 79, 80, 81]. Exploratory work was carried out *in vitro* with rat thalamocortical brain slices using a free electron laser that allowed investigation of multiple wavelengths. Larger spot sizes were found to require a lower radiant exposure for stimulation, as stimulating a larger area requires fewer neurons to react to the stimulus per unit area compared to a smaller spot size [79].

The efficacy of INS to stimulate the brain *in vivo* has since been demonstrated in two studies [65, 80]. The somatosensory cortex of rats and primary visual cortex of macaque monkeys have shown to respond to INS and have shown strong spatial isolation.

### 3.3. INS in the Auditory System

Electrical stimulation of the cochlea has produced one of the world's most successful bionic devices [32]. One current limitation of the implant is the spread of electrical current through the tissue and the perilymph, reducing the spatial selectivity that can be achieved with this stimulation modality [3]. The improved spatial selectivity of INS potentially makes it a very attractive technique for stimulation of nerves in the cochlea and consequently the area has seen much research.

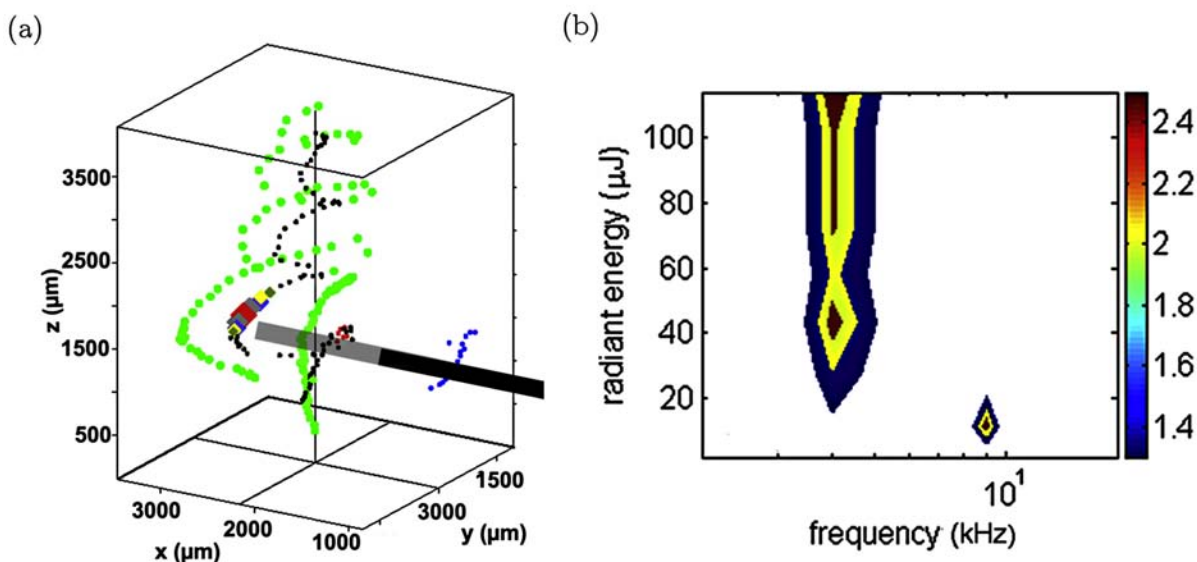
INS of the cochlea was first performed in gerbils by Izzo *et al.* [66]. Optical radiation from a Ho:YAG laser ( $\lambda = 2120$  nm) targeting the modiolus was delivered to the cochlea by a  $100\ \mu\text{m}$  core diameter fibre, with pulse durations of  $250\ \mu\text{s}$  at 2 Hz and a distance of  $0 - 500\ \mu\text{m}$  from the target. Compound action potentials (CAPs) were observed in response to the laser pulses in both normal hearing and deafened animals in which the acoustic threshold had increased by approximately 40 dB. As a result of the deafening, the authors suggest that the interaction occurs directly with the nerves and is not mediated by an optoacoustic effect involving the hair cells. No evidence of neural damage was observed during the stimulation, suggesting that stimulation at this rate was safe for the duration of the experiment (6 hrs).

While the work of Izzo *et al.* [66] demonstrated that the auditory system responded to the optical stimulus, it did not demonstrate the proposed advantage of improved spatial selectivity, or limiting stimulation of the auditory system to a smaller frequency range than is possible with electrical stimulation. Spatial selectivity has been investigated with a range of techniques, including: fluorescent staining with c-FOS to identify excited neurons [82]; inferior colliculus (ICC) recordings which identify the localisation of signals to

different neural pathways [83]; micro-CT reconstructions of the cochlea [84] (as shown in Fig. 1) and acoustic tone masking [85]. Overall, these results showed similar localisation of neural activity for both INS and acoustic tone pips, although some results point to stimulation on the far side of the cochlea rather than neurons directly in front of the optical fibre [83].

A range of laser parameters have been used for INS in the cochlea and a number of studies have been performed on the effect of varying the pulse length [67-69, 74, 86] and wavelength [55, 67, 68, 74, 87] used to stimulate the neurons. The optimal wavelength depends on the fibre position, but generally a wavelength with penetration depth of greater than the distance between the neurons and the fibre is most effective. These and other results have shown that the cochlea responds to infrared pulses with roughly an order of magnitude less energy than other targets for INS (See Table 1 for an overview), although this is more likely to be attributed to a more sensitised endpoint than to an inherent difference in neural sensitivity to INS in the cochlea. Pulse lengths of  $35\ \mu\text{s}$  to  $1600\ \mu\text{s}$  have been studied, with the required energy per pulse scaling with pulse length (i.e. displaying a power dependent response). In some studies, pulse durations below  $100\ \mu\text{s}$  required the same total energy as the  $100\ \mu\text{s}$  pulses but a higher peak power [67, 68]. However, this result has not been found in all studies [69].

As the rapid heating caused by INS laser pulses can generate an acoustic click [88], any results of INS in the cochlea require controls with deafened animals. A number of studies have demonstrated that a response can be generated when the cochlea has been acutely deafened [66, 69, 89]. Deafening techniques commonly used involve applying neomycin directly to the round window or cochleostomy and allowing it to perfuse through the cochlea. This technique



**Fig. (1).** (a) 3D reconstruction of a guinea pig cochlea during stimulation. Black cylinder shows the position of the optical fibre, black dots indicates the spiral ganglion neurons, green dots are the inner pillar feet. (See online for colour) (b) The spatial tuning curve obtained from recordings of the ICC, showing stimulation at 10.8 kHz and 16.1 kHz. Fig. reproduced from [84], with permission from Elsevier.

typically increases acoustic thresholds by at least 40 dB [69, 89]. Generally, these results have shown a small change in response between the normal hearing and acutely deafened cases. Chronically deafened animals have shown an increase in the energy required to generate a response [69], and with a sufficiently high concentration of neomycin no response to optical or electrical stimulation has been observed in some animals [69]. The increased threshold in chronically deafened animals was attributed to a reduction in the spiral ganglion neuron count (SGNs) [69], which would imply that a larger volume of neural tissue must be stimulated to generate the same response. Recently, further research has been performed using tone masking, to test whether there is any acoustic response to the laser. Results have shown that the laser interaction with the target neurons is not primarily acoustic [89].

Currently, only limited behavioural studies have been performed on animals. Matic *et al.* [90] chronically implanted cats with an optical fibre targeting the spiral ganglion neurons in the cochlea. During stimulation, behavioural observations were made. Cats were individually released and observed while the laser was actively stimulating and while it was off during stimulation the cats made more turns towards the implanted ear, suggesting perception of the laser pulses. However, proof of perception requires further experimentation to explicitly show that the animals perceive a sound.

Stimulation of the cochlear nucleus was demonstrated by Lee *et al.* [91]. They recorded optically evoked auditory brainstem responses from a 400  $\mu\text{m}$  core diameter fibre directed towards the cochlear nucleus. Unlike the results from the Richter group, this work targeted the central auditory neurons, rather than the more peripheral spiral ganglion neurons. They noted that the ABR recording was typical of that produced by acoustic stimulation and had a latency of 3 to 8 ms longer than that evoked by electrical stimulation in the same region. A follow up study found that after cutting the auditory nerve to deafen the animal, no response could be found from INS [92]. This suggests that the previous results may have been due to an acoustic artefact.

A hair cell mediated response to laser stimulation, or optoacoustic stimulation, has been demonstrated by Wenzel *et al.* [93]. In this work, green 532 nm laser light was delivered to the cochlea, generating an optoacoustic response. The laser pulses were 10 ns in duration with energies up to 23  $\mu\text{J}$  delivered by a 50  $\mu\text{m}$  core diameter fibre. The response in most animals saturated at approximately 15  $\mu\text{J}$  and the response was similar when stimulation was performed with the fibre inserted through the round window, or with the round window intact. No damage was observed with stimulation at  $E_{\text{pulse}} = 13 \mu\text{J}$  and a stimulation rate of 10 Hz for a duration of 30 mins. After deafening with kanamycin and ethacrynic acid, no response to the laser stimulation could be observed. These results suggest that the cochlea can respond to laser-induced acoustic events, although the response to optoacoustic sits in a greatly different pulse length and radiant exposure regime compared to INS in the cochlea.

Schultz *et al.* [94] showed that a range of wavelengths, from 400 nm to 2000 nm, can stimulate the cochlea with a nanosecond laser. In this regime, the response has a correlation between the water absorption or haemoglobin absorption of the wavelength. When the cochlea was deafened with neomycin no response to the laser was observed and no strong response has been observed in spiral ganglion neurons *in vitro* [95]. While this suggests that INS could be mediated through an optoacoustic mechanism, results from [66, 69] and [89] suggest that the response seen during INS is not dependent on functioning hair cells. It appears that further research is required to unravel the mechanisms behind these disparate findings for optical stimulation in the auditory system.

### 3.4. Safety of INS

As INS involves heating tissue, any long term use of this technique requires an understanding of the thresholds for damage and the safety margins required.

Studies on the safety margins [58] found that that the threshold to stimulate tissue and corresponding 95% confidence ranges did not overlap with the radiant exposures found to cause damage, when stimulation was performed at rates between 2 – 8 Hz. Damage was assessed by analysing histological samples for thermal lesions. Damage thresholds were found to be 0.7  $\text{J}\cdot\text{cm}^{-2}$  for a 1% probability of damage and 0.91  $\text{J}\cdot\text{cm}^{-2}$  for a 50% probability, when using histological analysis of exposed tissue. Additionally, higher pulse rates (5 – 8 Hz) were more likely to cause damage than 2 Hz. Damage from higher stimulation rates of 200 Hz have been studied in rodents and squirrel monkeys [96]. At these frequencies a damage threshold of 0.3–0.4  $\text{J}\cdot\text{cm}^{-2}$  was found, lower than that in the peripheral nerves. These results show that care will be needed to find the safe INS parameter space, especially at high frequencies.

Although thresholds for INS in the cochlea appear to be much lower than that required for other neural targets, maximising the usefulness of INS for implants may require high stimulation rate, on the order of several hundred Hz, to be comparable to electrical stimulation in cochlear implants [32]. To date, a number of studies focusing on the safety in both acute and chronic usage of INS in the cochlea have been performed [66, 67, 97, 98, 90]. Recent reports of acute experiments indicate that no damage occurs at stimulation rates of up to 250 Hz in a cat cochlea with a pulse energy 25  $\mu\text{J}$  over periods of up to 5 hours [98]. Cats with chronic implants have shown no evidence of damage at a stimulation rate of 200 Hz for 6 hours per day over 30 days [90]. Overall, these results are encouraging for the use of INS for long term implants.

An Arrhenius model for describing the kinetics of cellular damage from INS due to heat shocks to neurons and neuroglia was developed [99]. Experimental data from *in vitro* stimulation of rat astrocytes from a 1550 nm laser source provided the data on cell survival while temperatures were calculated with a model [100]. Combining these results

with a finite element model of heating, such as [101, 74, 100], may allow for predictions of damage with different pulse durations and stimulation frequencies.

### 3.5. Mechanisms Behind INS

The mechanisms behind INS have been the topic of some discussion in the literature and a number of potential mechanisms have been identified which may contribute to the response observed from laser exposure.

Mechanisms of INS were first investigated by [54] Wells *et al.*, using a Ho:YAG laser ( $\lambda = 2120$  nm), free electron laser ( $\lambda = 2100$  nm) and diode laser ( $\lambda = 1870$  nm) to stimulate the rat sciatic nerve. They found that a photothermal interaction due to water absorption was the most likely mechanism behind INS, rather than photomechanical pressure waves or photochemical mechanisms. As the pulses used are well below stress confinement there is not a large pressure wave generated, reducing the possibility of a photomechanical mechanism. Photochemical effects were ruled out as direct photochemistry requires a photon energy greater than that found at the wavelength used for INS ( $< 0.1$  eV). Additionally, transient tissue heating or a thermal gradient with respect to time was found to be required in order to achieve neural activation, as simply heating the tissue does not generate a response. The authors speculated that heat-sensitive ion channels or a change in conductance of ion channels may be behind the response to the transient heating of neurons. The importance of transient heating was also shown by Rajguru *et al.* [102], who investigated INS of adult oyster toadfish *crista ampullaris*. Exposure to 1962 nm IR radiation resulted in an increase in the firing rate, while the application of indirect heat did not.

Following the proposal of Wells *et al.* [54] that heat-sensitive ion channels may be a mechanism behind INS, the heat-sensitive vanilloid subfamily of TRP channels (TRPV) [103, 104] have been the subject of some research in the literature [105-107]. The TRPV channels, along with the TRPM subfamily, have the potential to detect changes in temperature from 10 to 50°C [104, 108]. Different TRPV channels activate at different temperatures,  $>25^\circ\text{C}$  for TRPV4 [109],  $>31^\circ\text{C}$  for TRPV3,  $>43^\circ\text{C}$  for TRPV1,  $>52^\circ\text{C}$  for TRPV2 [110]. Additionally, this temperature dependence is affected by the membrane voltage [111]. Expression of TRPV channels varies in different tissue [104] and could potentially explain some variations between different neural targets. Additionally, Rhee *et al.* [112] exposed dissociated neurons from the vagus nerve of rats to laser stimulation and measured the change in intracellular  $\text{Ca}^{2+}$  concentrations. When a TRPV1 channel blocker, capsazepine was added, the laser exposure no longer generated a  $\text{Ca}^{2+}$  transient from an influx of  $\text{Ca}^{2+}$ .

Further work on TRPV channels was reported by Albert *et al.* [107]. The response of retinal and vestibular ganglion cells to  $\lambda = 1875$  nm light was examined using whole cell patch clamp recording. The influence of TRPV channels was

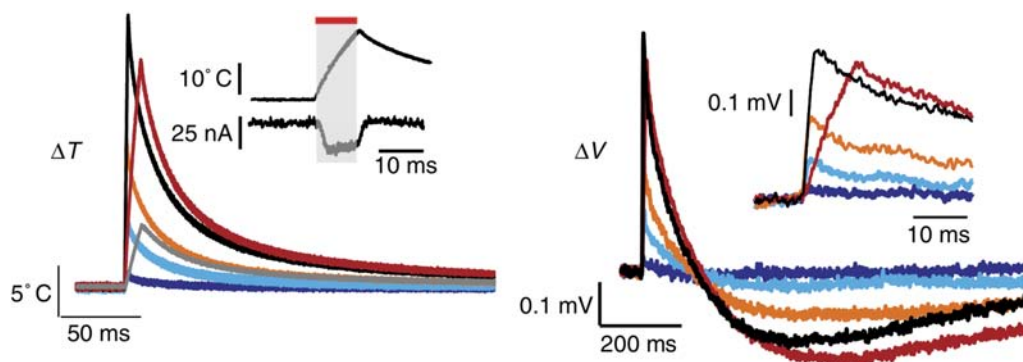
determined by adding various channel blockers, to remove their contribution to the response. When the TRPV4 channel was blocked, a response to the laser could no longer be produced, suggesting that TRPV4 channels play a role in neural activation from INS.

Heating of neurons may also accelerate other processes in the cell membrane as the reaction rate increases with temperature. Recently, heating of hippocampal neurons with 808 nm light has been shown to accelerate  $\text{Na}^+$  current kinetics [113]. Similarly, the response of intracellular calcium due to heating from INS has been investigated by [114]. A  $\lambda = 1862$  nm diode laser was used to stimulate rat ventricular cardiomyocytes and the resultant intracellular calcium wave was imaged using a confocal microscope. The authors found that TRPV channel blockers 2-APB and Ruthenium Red blocked the intracellular release in response to INS. However, CGP-37157, an inhibitor of mitochondrial  $\text{Na}^+/\text{Ca}^{2+}$  exchange (mNCX) also blocked the response observed due to exposure to IR. The authors concluded that the mitochondria are the primary facilitator of the IR-evoked  $\text{Ca}^{2+}$  transients.

Recently Shapiro *et al.* [56] have shown that the membrane capacitance varies reversibly during rapid heating, such as occurs during exposure to INS. The authors propose that INS is mediated by this shift in capacitance as it causes a change in membrane voltage that can initiate an action potential. Additionally the authors used heavy water ( $\text{D}_2\text{O}$ ) to demonstrate the role of water as the primary chromophore absorbing laser irradiation during INS. Heavy water has approximately 20% the absorption coefficient of normal water at the wavelength investigated (1889 nm). When standard water was replaced by heavy water, the observed response reduced by 80%, demonstrating that light is primarily absorbed by water during INS. Oocytes, HEK cells and artificial lipid bilayers were exposed to 1869 nm (HEK cells) or 1889 nm laser light (oocyte and bilayers) and the response was recorded with a patch clamp.

Recordings of the temperature shift using micropipette resistance measurements displayed a similar shape to the change in membrane voltage, as shown in the example in (Fig. 2). The observed change was consistent with a model of double layer capacitance and shows how the membrane voltage changes proportionally to the temperature. The capacitive mechanism was further investigated by Liu *et al.* [115], which confirmed the importance of the rate of change in temperature, rather than the absolute temperature change. However, the authors argue that the magnitude of the voltage change due to capacitance is unlikely to act as an excitatory stimulus, except for the most voltage sensitive cells [115].

Peterson *et al.* [116] applied the changes in membrane capacitance observed by Shapiro *et al.* [56] for the simulation of neurons to assess whether this mechanism would be adequate to explain the response observed *in vivo* and if myelination would change the response. They found that the capacitive mechanism was unable to activate neurons on its own and is unlikely to be the only mechanism



**Fig. (2).** Example of temperature measurement (left) and change in cell membrane potential (right) during and after exposure to INS. Adapted by permission from Macmillan Publishers Ltd: Nature Communications [56], copyright (2012).

behind INS. Additionally, they found that smaller fibres would have a lower activation threshold.

Roth *et al.* [117] found that cells exposed to nanosecond pulsed electric fields created nanopores in the cellular membrane, leading to an influx of  $Ca^{2+}$ . The authors speculate that the response seen due to exposure to INS may also be due to the formation of temporary, subtle disturbances in the cell membrane, based on the similarity in observed electrophysiological response.

Despite considerable progress towards understanding the mechanism behind INS, the details of the process have not yet been fully explained and the topic is still subject to further research. However, the current evidence points to changes in membrane capacitance underlying the primary response observed, with temperature dependent effects on transmembrane ion channels and TRPV channels playing an additional modulatory role.

## 4. EMERGING TECHNIQUES

### 4.1. Combined Optical and Electrical Stimulation

One of the potential disadvantages of INS is the heat deposited in tissue, especially at high repetition rates [118, 101]. Any reduction in the total energy delivered to the tissue would be advantageous for the development of implants both from a tissue heating consideration as well as from a laser device design point of view. Additionally, the current laser sources consume significantly more power than existing electrical implants such as the cochlear implant. Minimising total energy consumption will be important in developing portable and compact implantable stimulation systems. Duke *et al.* [70] proposed using sub-threshold electrical depolarisation to reduce the optical energy required for stimulation. An initial investigation [70] found a three fold reduction in the optical energy when the electrical stimulus was 90% of threshold. These results showed the potential of electrical-optical hybrid stimulation. The strongest response was observed when the two pulses ended simultaneously. Additionally, the authors found that the relationship between increasing electrical current and required optical energy did not follow a linear relationship, implying that the two

stimulation modalities do not function by the same mechanism.

Electrical-optical hybrid stimulation was further investigated in [71]. Here a rat sciatic nerve and *Aplysia californica* buccal nerve were stimulated using either a 2120 nm Ho:YAG laser or a 1875 nm diode laser and standard electrodes. For the *Aplysia* experiments, the light was coupled into a 100  $\mu\text{m}$  or 200  $\mu\text{m}$  core diameter fibre to match the nerve size; while for the rat sciatic nerve, the light was delivered by a 400  $\mu\text{m}$  or 600  $\mu\text{m}$  core diameter fibre as the nerve trunk is larger in this model. Both the temporal and spatial parameters of the optical and electrical stimuli were investigated. The authors found a strong spatial dependence on the location of the optical stimulus relative to the electrodes, that excitation could only be performed when stimulated near the cathode and that the excitable area was larger when the electrodes were configured transverse to the nerve than parallel. Additionally, in *Aplysia*, increasing the optical radiant exposure could cause inhibition of the nerve, even when the electrical pulse was set to 110% of the threshold. The authors also noted that the choice of laser source greatly affected the performance of hybrid stimulation: although both wavelengths had the same absorption coefficient in water, the Ho:YAG showed greater reproducibility than the diode.

The muscular response due to exposure of neurons to electrical-optical hybrid stimulation was investigated in [77]. Here a rat sciatic nerve was exposed to electrical and hybrid electrical-optical stimulation and the force generated in the plantarflexor muscles was measured in response to the different stimulation modalities and parameters. The optical stimulus was delivered by a 400  $\mu\text{m}$  core diameter optical fibre connected to a diode laser ( $\lambda = 1875 \text{ nm}$ ), the light was delivered through a Sylgard nerve cuff which was found to have 93% transmission at the wavelength used. The nerve was stimulated at rates of 15 and 20 Hz for a period of 1 second. Unlike electrical stimulation, responses to hybrid stimulation increased during a pulse train reaching a plateau by the 20th pulse. Additionally, an isolated optical stimulus before the hybrid pulse trains showed an increase in force generated. These results suggest that an increase in the



baseline temperature of the nerves is a contributing factor to the response observed during hybrid stimulation.

Hybrid electrical-optical stimulation has been used to show inhibition of electrical responses *in vivo* [72, 119] and spontaneous neural activity *in vitro* [100]. Use of a pulsed INS laser reversibly inhibited action potential creation and blocked action potential conduction in both rat sciatic nerve and *Aplysia* buccal nerve [119]. Infrared neural suppression is likely due to thermal block, which has previously been observed [120]. However, the technique presented allowed for suppression with a spatial resolution as small as 100  $\mu\text{m}$  and temporal resolution on the order of  $\sim 500$   $\mu\text{s}$ . Use of this technique may allow non-invasive investigation of neurological tissue and could potentially be applied to treating neurological disorders.

Overall, the work on electrical-optical hybrid stimulation suggests that the optical energy requirements could be reduced by up to 90%, without a reduction in spatial localisation compared to INS alone. However, this reduction is dependent upon electrode position relative to the target neurons and some recent research [72, 100] suggests that this process may not be applicable to all neural targets.

#### 4.2. Nanoparticle Enhanced INS

An alternative technique to enhance INS is by adding light absorbing materials to the tissue. The use of nanoparticles to stimulate of neurons under exposure to magnetic fields has previously been demonstrated [121] and now researchers are exploring the potential of using light to stimulate neurons via heating of nanoparticles [122, 123]. If nanoparticles are selected to absorb strongly at wavelengths that are weakly absorbed by water and tissue, then the absorption can potentially be localised near the targeted nerves. Enhancing absorption at the neurons has the dual benefits of reducing the power required for stimulation and allowing for wavelengths with increased penetration depth. Farah *et al.* [122] introduced micron scale photo-absorbing particles (iron oxide) to cultured rat cortical cells. The light was holographically patterned onto the target cells, reducing the total exposure of light to the culture. Using the combination of photo-absorbers and patterned light, significant reductions in the energy required to achieve stimulation were observed. Additionally, this technique could allow for the use of wavelengths that are not strongly absorbed by water, thus allowing greater penetration depths to be obtained.

The use of gold nanorods in cells as a selective absorber of near infrared light has also been investigated for *in vitro* neural stimulation [123, 124]. Initial work on cells cultured with gold nanorods demonstrated intracellular  $\text{Ca}^{2+}$  transients in response to laser light at the plasmon resonance wavelength of the gold nanorods [124], similar to cells exposed to INS [114]. Follow-up work found that action potentials could be evoked *in vitro* with in spiral ganglion neurons cultured with gold NRs under exposure to 780 nm light [123]. This technique used approximately two orders of magnitude less energy than INS. While these approaches

based on selective absorbers appear promising, methods for cell specific targeting of the nanoparticles may be necessary for practical applications. Obviously by introducing an exogenous substance to the target tissue this approach loses one of the main benefits of INS.

#### 4.3. Optoelectric Neural Stimulation

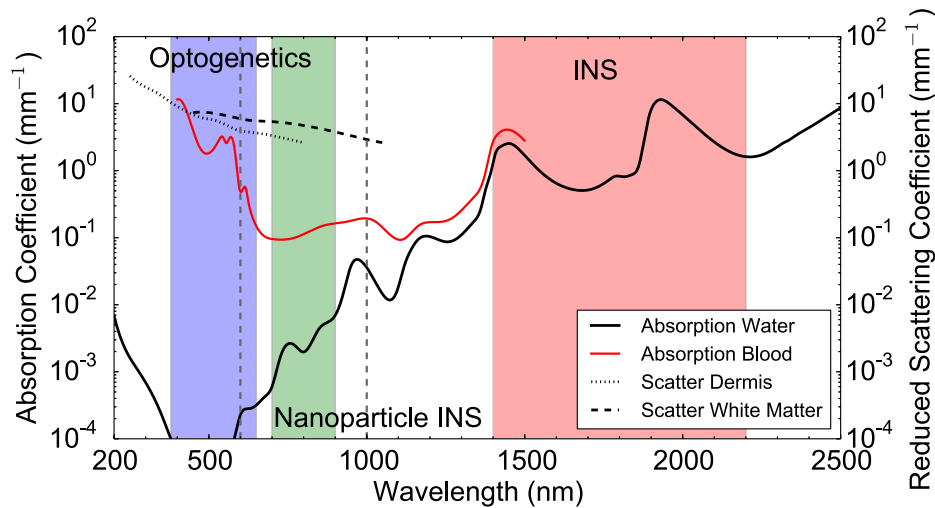
Optoelectric neural stimulation is another novel technique to stimulate neurons using light [125]. This technique uses photoactive nanoparticles or surfaces which generate an electric field in response to exposure to light, resulting in localised neural stimulation. Optoelectric neural stimulation may potentially combine the tight spatial localisation of light with the general applicability of electrical stimulation. A range of photoactive materials are being developed with the aim of neural stimulation, including quantum dots [126, 127], photoconductive silicon [128], organic polymers [129, 130], and carbon nanotubes [125, 131]. Many of these techniques are particularly attractive for application in to a retinal prosthesis, where an optical switch at the neurons would greatly simplify the implementation of any retinal prosthesis.

Quantum dots are semiconducting nanoparticles with fluorescent and other optoelectronic properties [132]. These properties allow for the generation of free electrons which can trigger action potentials in neurons [126]. Current limitations include the challenges with biocompatibility [133] of these molecules and improving efficiency to reduce the optical power required [125].

Photoconductive silicon has been demonstrated as a substrate that can generate electric charge to stimulate neurons under exposure to light [128, 134]. However, silicon is limited by its rigidity and biocompatibility, thus potentially limiting its use as a prosthetic platform [135]. Conductive polymers may overcome many of these limitations as an optoelectronic material [129, 130, 136, 137, 138, 139], although challenges in ensuring biocompatibility and stability of these polymers also remain to be resolved [125, 129].

### 5. LIGHT DELIVERY TECHNIQUES

Delivery of light to the target neurons is challenge common to all optical stimulation techniques. For *in vitro* work, light is commonly delivered via optical fibre or microscope but for *in vivo* work this becomes more challenging. Different forms of optical stimulation have different challenges. Genetic and caged molecule techniques typically use lower power, but shorter wavelengths where scattering and absorption in tissue is high. Conversely, infrared techniques require delivery of higher power levels and have to contend with strong absorption in water. Some of the emerging techniques that respond to wavelength in the range of 800–1000 nm may have the least challenges with tissue or water interfering with delivery of light to the target neurons. This difference in absorption and scattering in wavelengths used for optical stimulation is shown in (Fig. 3).



**Fig. (3).** Absorption coefficient in water [140] and oxygenated blood (5% haematocrit) [141]. Reduced scattering coefficient in dermis [142] and white matter [142] over the wavelength range of 200 nm to 2500 nm. Wavelength ranges commonly used for Optogenetics (380 – 650 nm), INS (1400 – 2200 nm) and Nanoparticle Enhanced INS (700 – 900 nm) are highlighted. Vertical dashed grey lines show the therapeutic window where absorption and scattering is relatively low.

### 5.1. Optical Waveguides

Optical fibres have provided the standard delivery mechanism for many optical stimulation experiments, as they are well-established optical components and relatively easy to use. A variety of standard silica core fibres have been used with a range of diameters, including for example, 600  $\mu\text{m}$  [54, 57], 400  $\mu\text{m}$  [56, 77], 200  $\mu\text{m}$  [69] and 100  $\mu\text{m}$  [66, 107]. Optical fibres are waveguides that have a core and cladding, most commonly constructed of silica. The refractive index of the core is slightly higher than that of the cladding, so that the light is reflected by total internal reflection if the angle of incidence exceeds the critical angle. Light is only coupled into the fibre if it falls within the acceptance cone is, which is generally often described by the numerical aperture (NA). A large numerical aperture has a wider acceptance cone. Fibres are typically protected from the environment with a number of jacket or buffer layers. For typical optical stimulation experiments, these protective layers are removed near the emitting end of the fibre. For further details on optical fibres, readers are referred to the comprehensive text on waveguides by Synder and Love [143].

Optical fibres have been implanted *in vivo* to allow optical control of neurons in free animals [33, 90, 144]. Aravanis *et al.* [33] delivered 473 nm light through an optical fibre implanted in a rat to excite neurons transfected with ChR2 in the control motor cortex of the CNS. Stimulating the neurons with blue light created an increase in whisker deflection, indicating stimulation in the motor cortex. A more integrated approach was taken by Towne *et al.* [144] where an optical fibre and PDMS optical nerve cuff on the sciatic nerve was implanted in rats [144]. Delivering light to the nerve demonstrated control of muscles in freely moving rats. A fully portable optical delivery system has been demonstrated by Matic *et al.* [90], where an optical fibre was implanted in the cochlea of cats with miniaturised

laser system mounted in a backpack. This enabled the animal to move feely, while its response to stimulation could be monitored.

A laparoscopic probe designed to deliver a collimated top hat beam profile to allow uniform illumination of the cavernous nerves was developed in [145]. The probe delivered a beam with a diameter of 1 mm, when coupled to a thulium fiber laser ( $\lambda = 1870$  nm). This probe showed similar results to those from previous work using a fibre [61] and may allow easier targeting of nerves for INS. This probe was adapted [146] to provide temperature monitoring and feedback with the continuous wave stimulation technique [75]. An IR sensor monitored the temperature on the surface of the nerve and controlled the laser power, allowing a fast ramp up in temperature without increasing to levels that may cause damage. This simplified the number of parameters to be optimised to achieve stimulation and allowed a faster response.

To enable an optically based implant to stimulate the cochlea, work has been performed to develop a waveguide-based optical delivery system [147]. Using optical fibres with a cladding diameter of 25  $\mu\text{m}$ , bundles of eight fibres could be inserted up to 20 mm into the cochlea without breaking. Insertion forces were similar to those measured with conventional cochlear implants. These results suggest that an optically based cochlear implant may be feasible, although many challenges such as beam targeting remain. Regardless of the application, there are fundamental limitations of using fibre optics in terms of mechanical stiffness and the ability to sustain sharp bends [148], in particular when compared to coiled wire electrodes. These engineering challenges must be address before optical neural interfaces can become a reality.

Optogenetics has also generated interest in advanced light delivery techniques. Typically optogenetics requires a lower laser power level and therefore can accept reduced

coupling efficiency and greater losses. Zorzos *et al.* [149, 150] reported custom fabricated waveguides, capable of delivering light in a 3D pattern in tissue. Holographic patterning of light onto neural tissue was demonstrated by [122, 151]. This technique uses a spatial light modulator to deliver light to individual neurons that have been photo-sensitised with optogenetic treatments or by using photo-absorbers [146].

## 5.2. Arrayed Light Sources

While fibres have been able to deliver light to a localised area and are able to display a significant improvement over electrical stimulation for some applications [83], there is interest in alternative delivery techniques that are able to deliver light closer to the nerves or to multiple nerves from a single implant for both INS and other optically-mediated nerve stimulation techniques [152].

An implantable multiwaveguide device has been the topic of some research [153]. Abaya *et al.* [154] developed a Utah slanted optrode array (USOA), similar to the Utah slanted electrode array, which has allowed for more specific electrical stimulation of neurons in nerve trunks than was achievable with traditional electrodes [155]. The USOA is aimed at delivering light to deeper tissue and to allow an even more selective stimulation of nerve trunks. The USOA is micromachined from a silicon wafer and etched to form narrow shanks 0.5 mm to 1.5 mm in length. Transmission efficiency varied between 2 - 10% depending on the diameter of the fibre used to couple light into the array. Losses in coupling arise due to Fresnel reflection between the different interfaces, which are increased by the high refractive index of silicon compared to glass, and losses due to the tapering of the tips. The Utah slanted optrode array was improved upon in [156], which reported a 3D silica optrode array with non-tapering tips. An example of the fabricated array is shown in (Fig. 4). This resulted in a large increase in fibre-to-nerve coupling efficiency of up to 70% and significantly reduced shank transmission losses in the shank.

Another approach to deliver light to neurons is by developing microscale emitters and positioning or implanting them in close proximity to the target neurons

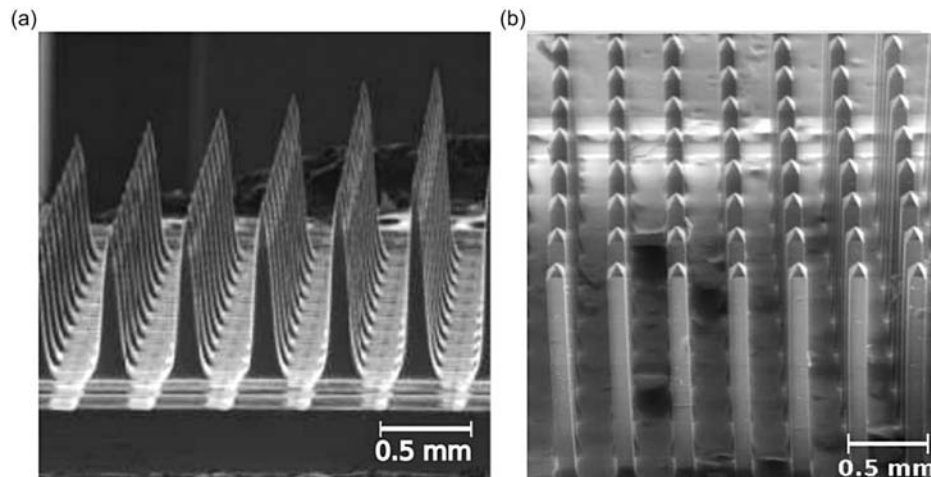
[157-159]. Poher *et al.* [157] fabricated a 64×64 matrix of 20 μm micro-LEDs, which were able to photostimulate sensitised neurons. This fabrication approach has been taken up by other researchers, including fabrication of 15 LED arrays suitable for implantation in a ChR2 sensitive cochlea [158] and vertical cavity surface emitting lasers to provide high intensity infrared light for INS [159].

## 5.3. Modelling of Light Delivery and Heating

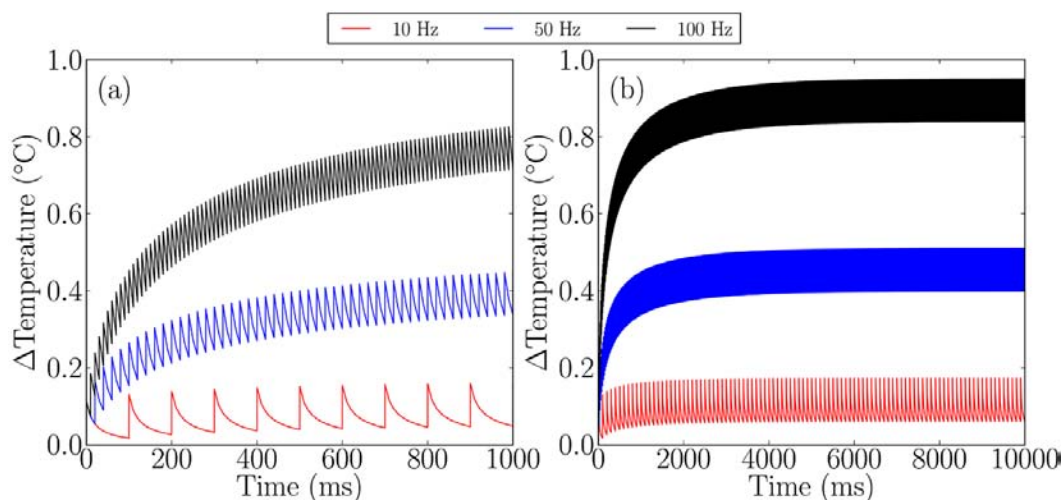
Recently, both analytical and numerical models have been developed to assist in understanding the flow of heat during INS, in the cochlea and *in vitro* models [55, 74, 101, 86, 100]. As INS depends on a thermal gradient in time [54, 56], understanding the heat distribution in tissue may help to optimise the process and assist in emitter design. Modelling may also be to provide detailed thermal information for *in vitro* studies aimed at uncovering the mechanisms behind INS.

Thompson *et al.* presented a combined numerical Monte Carlo and finite element model that allows the prediction of temperature increases from a single pulse [55, 74] or from a pulse train and multiple emitters [101], as shown in (Fig. 5). The model compares well with experimental measurements of temperature from laser heating [55, 74] and predicts temperature increases of 0.1°C for INS in the cochlea and on the order of 1°C to 10°C for other targets. The model shows where previously used “rules of thumb” are valid and where a more detailed approach is required.

A numerical multiphysics model of heating during INS *in vitro* was developed by Liljemalm *et al.* [100]. Using an optical fibre with 200 μm core diameter and NA = 0.39, wavelength of 1550 nm, laser power of 300 mW and pulse length of 20 ms, the model predicted a temperature increase of 13.7°C at the centre of the beam, 300 μm from the fibre emitter. When using multiple pulses at 10 Hz, the peak temperature increased by a further 1.7°C, stabilising after four pulses. Results from the model compared well with local temperature measurements using changes in current in a micropipette.



**Fig. (4).** SEM image of (a) silicon Utah slant optrode array and (b) glass optrode array reproduced from [153], with permission from SPIE.



**Fig. (5).** Heating from different stimulation rates with INS, when using a fibre with core diameter of 200  $\mu\text{m}$  and NA = 0.22, with a wavelength of 1850 nm and pulse energy of 25  $\mu\text{J}$ . **a)** Over 1 second **b)** over 10 seconds. Reproduced from [160].

Norton *et al.* [86] developed a Green's function analytical model of thermal changes during INS. Rather than investigate temperature distributions, the model was applied to understanding what thermal changes are required to activate neurons, specifically the cochlea. The authors hypothesised that two thermal criteria are required for neural activation: a minimum temperature increase  $T_c$ ; and a minimum temperature rate of change  $dT_c/dt$ . By optimising these criteria, a pulse length can be found that satisfies both conditions, thus reducing wasted energy. Experimental *in vivo* data of INS in the cat cochlea used in combination with a CAP growth function to give values of  $T_c = 0.8\text{m}^\circ\text{C}$  and  $dT_c/dt = 15.1\text{ }^\circ\text{C}\cdot\text{s}^{-1}$  for the thermal criteria and an optimal pulse length of 53  $\mu\text{s}$ . Further use of this approach to determine optimal pulse lengths may assist in developing more efficient INS implants.

Monte Carlo models have also been applied to understand light distributions in tissue during optogenetic stimulation [9, 33, 161]. Most current opsins used for optogenetic stimulation respond at a much shorter wavelength than used in INS, where scattering in the tissue dominates over absorption. A wavelength of 473 nm, which activates commonly used opsins, has less than 20% light transmission through 500  $\mu\text{m}$  of brain tissue when using a fibre core diameter of 200  $\mu\text{m}$  and NA = 0.37. If light with a wavelength of 594 nm is used instead, transmission increases to 30% due to the decreased scattering at these wavelengths [9].

## 6. CONCLUSION

The accelerated development of optical nerve stimulation techniques over the past decade or so has provided neuroscientists with a powerful new range of tools for controlling neuronal activity. While no one tool currently offers a complete solution to all of the requirements for

manipulating neuronal function, each of the techniques has some specific advantages. For example, the two-photon uncaging of glutamate allows a high degree of spatial localization and precise timing of stimulation events. However, the glutamate is gradually depleted by repeated photolysis, so this method is less effective for prolonged stimulation or high repetition rates. Likewise, genetically targeted ChR2 is attractive for controlling and mapping neural circuits *in vivo*, but requires suitable promoters for specific cell types and has limited depth resolution due to the use of visible light.

Infrared neural stimulation is attractive for direct activation of nerves via the transient heating of water, without any need for tissue modification. However, the technique remains relatively poorly understood, requires high light intensities and has demonstrated limited localisation and depth penetration due to the prevalence of water in all tissues. Emerging techniques based on extrinsic absorbers may overcome some of the deficiencies of infrared stimulation, but at the cost of introducing foreign materials to the nervous system. Therefore, given the undoubted attractions of optical stimulation, the remaining limitations will certainly lead to improvements in the current tools and the development of additional novel techniques. In particular, the development of a wider range of small molecule photoswitches promises to deliver great flexibility in controlling the activity of specific ion channels or receptors. This capability will provide new insights into the role of those targets in normal neuronal function.

Further progress can also be anticipated in terms of delivering light to the nervous system. Clearly those techniques that rely on near infrared light in the transparency window of tissue, such as two photon uncaging and gold nanoparticles, will have an advantage in terms of *in vivo* delivery. However, the need for implantable arrays of light sources or emitters will remain for many important targets, including the brain. Multiple active light sources will allow

synchronous recruitment of multiple neuronal targets, while holographic illumination systems can potentially also provide simultaneous access to a large number of stimulation sites. These efforts will be facilitated by modelling of light propagation and interactions with neuronal tissue, although there is a need for more detailed data on the optical properties of biological tissues across the entire wavelength range of interest.

Indeed as novel photonic technologies are developed more and more of these emerging tools will become available to neuroscientist and clinicians. Further progress in these various domains will eventually allow optical stimulation to deliver its full potential, driving important advances in our understanding of neural disorders, unravelling neural encoding and facilitating new interventions for sensory deficiencies and neural diseases. The future of this field is bright.

### CONFLICT OF INTEREST

The author confirms that this article have no conflict of interest.

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