Gold Nanorod-Enhanced Infrared Neural Stimulation

Chiara Paviolo, Alexander C Thompson, Jiawey Yong, William GA Brown, and Paul R Stoddart

Biotactical Engineering, Faculty of Science, Engineering and Technology, Swinburne University of Technology, Hawthorn, PO Box 218, Victoria 3122, Australia

E-mail: pstoddart@swin.edu.au

Abstract. Recent research has demonstrated that nerves can be stimulated by transient heating associated with the absorption of infrared light by water in the tissue. There is a great deal of interest in using this technique in neural prostheses, due to the potential for increased localization of the stimulus and minimization of contact with the tissue. However, thermal modelling suggests that the full benefits of increased localization may be reduced by cumulative heating effects when multiple stimulus sites and/or high repetition rates are used. Here we review recent *in vitro* and *in vivo* results suggesting that the transient heating associated with plasmon absorption in gold nanorods can also be used to stimulate nerves. In particular, patch clamp experiments on cultured spiral ganglion neurons exhibited action potentials when exposed to 780 nm light at the plasmon absorption peak, while the amplitude of compound action potentials in the rat sciatic nerve were increased by laser irradiation of gold nanorods in the vicinity of the plasma membrane. Similarly, calcium imaging studies of NG108 - 15 neuronal cells incubated with Au nanorods revealed an increased level of intracellular calcium activity synchronized with laser exposure. Given that the plasmon absorption peak of gold nanorods can be matched with the transparency window of biological tissues, these results demonstrate that nanorod absorbers hold great promise to enhance the process of infrared neural stimulation for future applications in neural prostheses and fundamental studies in neuroscience.

1. Introduction

Infrared light has been demonstrated as an alternative to traditional electrical methods for nerve stimulation [1, 2]. The technique of using infrared light to stimulate neurons has become known as 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 [1, 3, 4]. In addition, compared to optogenetic and caged molecule techniques [5], INS requires no modification of the target tissue, as it only relies upon the absorption of infrared light by water in the tissue [1].

The first demonstration of INS was in the rat sciatic nerve, using a free electron laser with wavelengths between 2 μm and 10 μm [2]. Since this initial demonstration, INS has been extended to a number of neural targets including the cochlea [6], the visual cortex [7], embryonic heart [8] and others [1]. From the wide wavelength range initially used, INS investigations have increasingly relied on the range from 1850 to 1870 nm. This wavelength range corresponds to water absorption of approximately $\mu_a \sim 2 - 3$ mm^{-1} and allows some trading off between penetration depth and absorption at the target [9]. Radiant exposures for INS are typically on the order of $0.1 - 1$ J $\cdot$ cm^{-2} [1] or pulse energies of approximately $125 - 1250$ μJ. Although the cochlea has shown thresholds in the $5-100$ mJ$\cdot$cm^{-2} range, the reasons for this increased sensitivity remain controversial, with recent work suggesting that an optoacoustic mechanism might be operative, rather than INS [10]. While the corresponding temperature increase of approximately 2 – 3 °C [11, 9] has been used safely at low pulse rates [12], stimulation at higher pulse rates has shown some evidence of damage [13].

A number of models have been developed to investigate the heating effects during INS [9, 14, 15, 16]. In general, they show that over typical distances between the emitter and the target neurons ($<$ 1 mm), water absorption is the dominant factor in determining the energy deposited for INS. In the time domain, for pulses of light delivered by multimode fibres, the models show that the heat remains well contained for up to 1 ms and that heating a smaller volume results in a faster return to initial temperature. When heat flow is taken into account, it is found that the temperature rise is higher for multiple pulses than for a single pulse, and is also increased if pulses are delivered concurrently to neighbouring sites [15]. Importantly, increasing the localization of heating reduces the maximum temperature at a given pulse rate, potentially allowing faster stimulation. Although the temperature increase from single pulses has been shown not to cause damage [12], some bionic applications, such as the cochlear implant [17] and bionic eye [18] require high stimulation rates in excess of 100 Hz together with multiple stimulation sites. For these applications, the build up of heat may limit the use of INS and reduce the spatial resolution that can be obtained. Recent work [4] has shown up to a 90% reduction in optical thresholds with a combined

electrical pulse which could potentially reduce energy requirements enough to allow the use of INS at higher pulse rates and expand the range of potential target systems.

In response to these challenges, researchers have recognized the potential to use extrinsic absorbers to produce more localized effects. Huang *et al.* [19] demonstrated the use of magnetic nanoparticles (NPs) to modulate heat sensitive TRPV1 channels under application of an RF magnetic field. Compared to optical techniques, this may allow for deeper penetration, as magnetic fields interact relatively weakly with tissue. However, the published results show responses over periods of seconds, rather than the millisecond durations required in bionic devices. A number of authors have investigated the use of photoactive materials to provide an interface between light and neurons [20]. These photoactive materials include semiconducting quantum dots which can be located near or within neurons targeted for stimulation [21]. While promising, there are concerns about toxicity of the quantum dots, which may require significant technological advances to resolve [22]. The use of light-absorbing particles has also been demonstrated: Migliori *et al.* [23] reported photothermal initiation of action potentials in *Hirudo verbana* neurons, using powdered carbon to absorb 50 ms duration pulses of 650 nm light with energies of 250 to 750 μJ. Recently, Farah *et al.* [24] demonstrated the stimulation of rat cortical neurons in culture with black microparticles. The use of microparticles in combination with holographic light patterning allowed stimulation with cellular precision at pulse durations of 100 μs and energies in the range of 1 μJ. The short pulses and tight spatial localization of absorption gave sub-millisecond thermal relaxation times, potentially allowing faster pulse repetitions than can safely be achieved by INS.

In the present paper, recent reports of neural stimulation due to absorption of light by gold nanorods are reviewed. After a brief summary of the known mechanisms of INS, the advantages of using gold nanorods for photothermal nerve stimulation are presented. This is followed by a summary of recent results and a discussion of the outlook for future progress in this field.

2. Mechanisms of INS

The precise mechanisms of INS are not fully understood at present; however, current evidence suggests that rapid heating of water in the tissue is the underlying driver [11, 25]. The secondary processes resulting from this heating include changes in membrane capacitance [25] and the activation of temperature-sensitive ion channels [26]. In the case of cochlear stimulation (see for example [6, 27, 28]), an optoacoustic effect may also play a role [10].

Shapiro and coworkers have demonstrated that rapid heating by infrared light causes changes in membrane capacitance, and proposed that this occurs by perturbing the distribution of charged particles in the electrical double layer immediately adjacent to the cell membrane [25]. These electrical double layers contribute to the effective membrane capacitance, with a magnitude that is related to the temperature-dependent distribution of charge within them. Taking the electrical double layers into account,

the total capacitance of a cell membrane can be modelled as the intrinsic capacitance of the lipid bilayer in series with the capacitances of the internal and external electrical double layers and therefore depends on temperature. Based on this model, Shapiro *et al.* were able to accurately predict the transmembane currents that were measured during infrared heating of artificial lipid bilayers.

Laser-induced transmembrane currents were also generated in HEK293 cells and *Xenopus laevis* oocytes. The responses in these cells were not sufficiently large to generate action potentials. However, by optimizing the method of stimulation it may be possible to generate enough depolarization to activate voltage-dependent ion channels and overcome this limitation. Furthermore, the highly general nature of this mechanism of stimulation means that it may be applicable to any type of cell and any mode of transient heating, providing ample justification for further exploration.

In cells containing temperature-sensitive ion channels, such as those of the TRPV family of ion channels, laser-induced changes in temperature can cause depolarization and subsequent generation of action potentials. This mechanism of INS was first described from experiments in retinal ganglion neurons and vestibular ganglion neurons *in vitro* in response to heating by 1875 nm light [26]. Pharmacological investigations found that a specific class of temperature-sensitive ion channels — TRPV4 channels — were responsible for the laser-induced action potentials and the following stimulation pathway was proposed: (*i*) activation of TRPV4 channels by heating leads to depolarization from the influx of calcium ions; (*ii*) this causes the activation of voltage-gated calcium channels, which enable additional calcium to enter the cell, depolarizing the membrane sufficiently to activate voltage-gated sodium channels; and (*iii*) action potentials are then generated in the usual way. While not as broadly applicable as the capacitance-based mechanism reported by Shapiro *et al.*, the heat-induced activation of TRPV4 channels (and possibly other channels from the TRPV family) could play a role in the stimulation of many cell types that contain TRPV channels, including targets in the brain, spinal cord and peripheral nerves [29].

Although the mechanisms of INS discussed here have been shown to be mediated by the absorption of laser light by water in the tissue, there is no fundamental reason that water should be the only absorber capable of generating the temperature changes required for INS-like responses. The broad and simple nature of this underlying process leaves open the possibility for substantial optimization by the addition of materials that exhibit strong absorption at wavelengths where water is transparent, as demonstrated in principle by Farah *et al.* [24] and Migliori *et al.* [23]. While adding an exogenous material to the tissue loses one of the advantages of INS, whereby no modification of the target tissue is required, it enables the use of wavelengths of light in the therapeutic window between 600 – 1200 nm [30]. This wavelength range has low absorption in water and hemoglobin, and reduced scattering compared to shorter wavelengths. In this way it is thought that the fundamental advantages of optically stimulating tissue, such as excellent spatial selectivity, can be realized, while at the same time minimizing potential risks and inefficiencies, such as nonspecific heating of intervening tissue in the path of

the illuminating light and allowing deeper penetration into tissue than is possible with more strongly absorbed or scattered wavelengths of light.

3. Gold Nanorods

The interest in Au NPs for biomedical photonics applications has increased considerably over the past decade due to their unusual optical properties [31]. When Au NPs are perturbed by an external light field in the visible or near infrared (NIR) domain, the conduction electrons move away from their equilibrium position, creating a resonant coherent oscillation called the localized surface plasmon resonance (LSPR) or plasmon excitation [32] (see Figure 1).

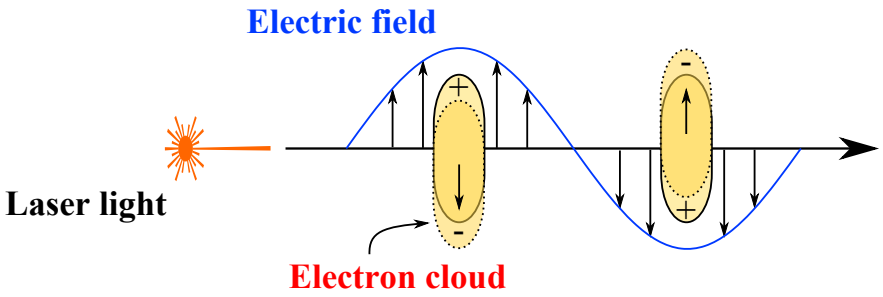

Figure 1. Schematic representation of the longitudinal plasmon oscillation in a gold nanorod (after [33]).

The optical properties of a variety of NP shapes, including spheres, rods, prisms and cubes have already been investigated [34]. Compared to other NPs, gold nanorods (Au NRs) have proven to be particularly useful, as they possess two distinct plasmon excitation bands, corresponding to the excitation of the short and long axes of the NRs. Modification ofthe nanorod aspect ratio allows an optical tunability that has provided several advantages for the use of Au NRs in biomedical photonics applications [35]. Au NRs can be prepared via wet chemical methods which provide a facile means for controlling the aspect ratio [36]. Moreover, the surface chemistry of Au NRs can be modified to improve their biocompatibility or binding affinity. Typical surface modification methods include polymer and/or silica coating. Figure 2 shows typical TEM images of unmodified and silica-coated Au NRs, both of which were prepared according to the methods used by Yong *et al.* [37] to achieve a peak absorption around 780 nm. In the context of neural stimulation, Au NRs allow a greater penetration depth through the near infrared (NIR) optical window of tissue and provide relatively favourable biocompatibility together with a wide range of surface functionalization options.

When Au NPs are exposed to a laser source, there are at least four distinct time regimes that can affect the result: *i*) low - energy femtosecond (ultrafast) laser pulses, *ii*) high - energy femtosecond laser pulses, *iii*) nanosecond laser pulses and *iv*) continuous irradiation. The irradiation of metal NPs with a femtosecond pulse leads to a rapid

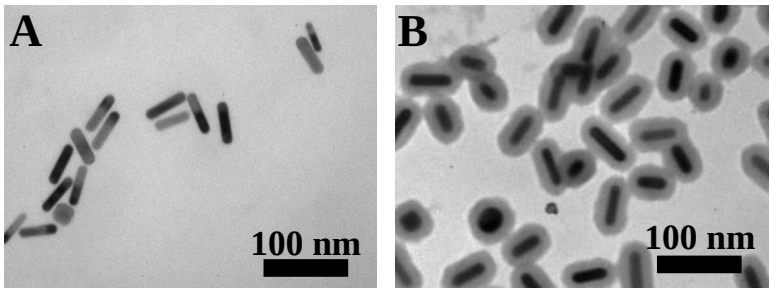

Figure 2. TEM images of (A) Au NRs, and (B) Au NRs with silica coating.

increase in electron energy. For low pulse energies, the ultrafast laser pulses raise the temperature of the NP lattice by only a few tens of degrees (depending on particle size, optical density and laser irradiance). In contrast, for high pulse energies, the temperature of the metal can be raised above its melting point, thus inducing a shape transition (e.g. nanorods transforming to nanospheres). In particular, melting occurs if the rate of heating of the lattice is faster than the rate of cooling. The time scale of heat dissipation from the particles to the surrounding environment is on the order of 100 ps to 10 ns, depending on the particle size, thermodynamic properties of the surrounding medium (thermal conductivity, heat capacity and solvent viscosity) and pulse energy [38, 39].

When nanosecond pulses are applied, the energy threshold for the complete melting of the NRs is effectively reduced due to surface diffusion, and normally only partial melting can occur at the surface of the particles [40, 41, 42]. In the case of continuous laser irradiation, the particles are constantly saturated, thus reducing their absorption efficiency and the overall photothermal energy conversion. No modifications in shape were detected, probably due to the low levels of laser irradiance that were used [43, 44]. This is consistent with the reported cases of gold nanorod-mediated neural stimulation [37, 60, 66]. It should be noted that all of the effects of laser irradiation on NPs depend not only on the laser parameters (wavelength, laser irradiance, pulse length), but also on the physical characteristics of the particles, such as size, concentration, distribution and surface chemistry. For example, Chen *et al.* demonstrated that the photothermal stability of Au NRs under nanosecond laser pulse irradiation was improved for silica-coated NRs [45].

3.1. Photothermal Effects

Near infared (NIR) laser light can be efficiently absorbed by Au NRs, provided that the incident wavelength matches the plasmon excitation wavelength. Heating of Au NRs occurs on the picosecond timescale, with the nanorods reaching thermal equilibrium with the surrounding environment within 100 ps to 1 ns [46]. The absorption coefficient of Au NRs is $\sim 10^8$ $M^{-1} \cdot mm^{-1}$ at 785 nm [47], whereas the absorption of NIR light by water is minimal (absorption coefficient = 0.05 mm^{-1}). Assuming a Au NR concentration of

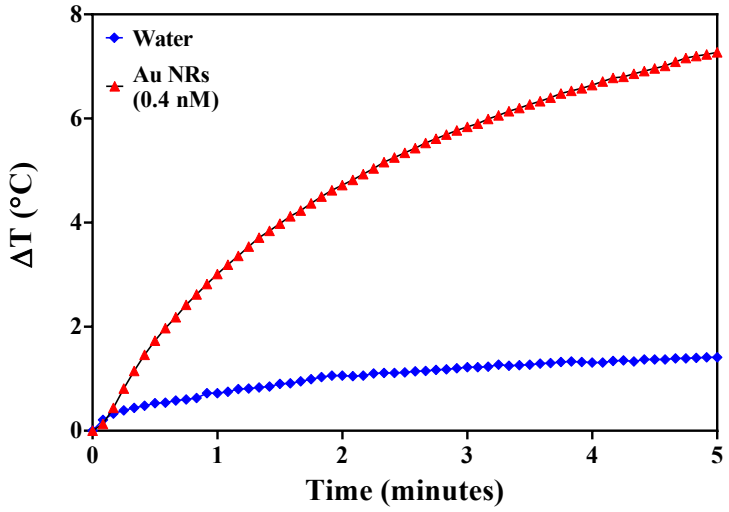

Figure 3. Laser-induced bulk heating of Au NRs suspended in water compared to pure water. The temperature change was measured experimentally relative to room temperature.

10 nM, this gives a 20-fold increase in absorption compared to pure water, potentially allowing light to be transmitted for $\sim$ 20 mm before interacting with the Au NRs.

To demonstrate the feasibility of NIR laser-induced heating of aqueous Au NRs and compare it with that of distilled water, the temperature of 100 μL samples was measured in narrow-bottom tubes with a K-type thermocouple under continuous exposure to a 780 nm laser. The optical density of the Au NRs at 780 nm was adjusted to $\sim$ 0.18, corresponding to a particle concentration of $\sim$ 0.4 nM. The laser irradiation was delivered vertically from an optical fiber at a distance of $\sim$ 2.4 mm from the sample surface. The heating curves of Au NRs and water are shown in Figure 3, where the temperature change in the nanorod suspension was seven times larger than that in pure water, after 5 minutes of continuous exposure with an irradiance of 25 W $\cdot$ cm^{-2}. The results suggest that at 780 nm, the water absorbs relatively weakly and thus exhibits poor photon-to-heat conversion compared to aqueous nanorods.

Compared to bulk heating, the temperature increase upon laser exposure is expected to be relatively fast and highly localized around the individual nanoparticles [46]. In cell culture experiments, the optimal pulse duration can be estimated by calculating the thermal relaxation time. If it is assumed that the nanoparticles are evenly distributed in a spherical cell and that a response is only evoked after heating the whole cell, the characteristic thermal relaxation time τ can be estimated from the cell size:

$$\tau = 0.00433\frac{4\pi r^2}{\alpha}, \tag{1}$$

where r is the radius of the sphere and α is the thermal diffusivity of the heated medium [48]. Taking the thermal diffusivity of water ($\alpha = 1.43 \times 10^{-6}$ m$^2 \cdot$ s^{-1}) and assuming a radius of 5 μm based on the typical size of a spiral ganglion neuron [49], a thermal

relaxation time of approximately 10 μs is found. This is much faster than the optimal pulse durations reported in INS, where tissue is heated in bulk, leading to thermal relaxation times on the order of milliseconds [11]. This simple analytical solution is similar to the results reported by Farah *et al.* [24]. While thermal relaxation times are likely to be greater in practice due to the heating of multiple neurons, this could potentialy provide an improvement on conventional INS, and may allow faster pulse repetitions to be used. A more detailed model of this process may allow further insights into optimizing the process of transient heating for neural stimulation with extrinsic absorbers.

3.2. Biocompatibility

When Au NPs are used in biological applications, they need to satisfy some basic requirements: they must not cause toxic or harmful effects, they need to be chemically and physically stable [35] and they require a pathway for functionalization with the desired surface chemistry [35, 50]. In the case of Au NRs, biocompatibility is particularly critical due the presence of a bilayer of a cationic surfactant (commonly CTAB) around the particles [39]. CTAB is known to induce cytotoxicity both *in vitro* [51] and *in vivo* [52] and to interfere with the surface hydration of the particles [53]. Depositing additional surface coatings has been one of the main strategies to reduce the negative effects caused by residual chemicals used during particle synthesis. The coating material should have the following favourable characteristics [35]:

- be easy to deposit;
- be controllable in terms of coating thickness;
- have a negligible effect on the LSPR;
- be chemically and physically inert;
- be optically transparent to the excitation and emission light;
- be nontoxic and biocompatible;
- be easily modifiable with functional groups for further biological conjugation.

It has been found that the effect of CTAB can be efficiently masked in NG108 - 15 neuronal cells by the addition of a polyelectrolyte layer (poly(styrene sulphonate)) or a silica shell to the Au NRs [54]. The cells showed good preservation of proliferation and cell membrane integrity after incubation with the coated particles. Importantly, it was observed that these nanoparticles could also induce neurite formation in NG108 - 15 neuronal cells after four days of culture [54]. Despite these promising results, it should be noted that the addition of a single polyelectrolyte layer may not entirely stop the release of molecules from the surface of the particles after laser irradiation [44].

The lack of severe cytotoxicity raises the question of whether the NPs are internalized by the cells or excluded by the cell membrane. In the majority of biological studies involving NPs, it has been observed that particle uptake is the primary cellular reaction. Chithrani *et al.* showed in 2006 that NP uptake was mediated via

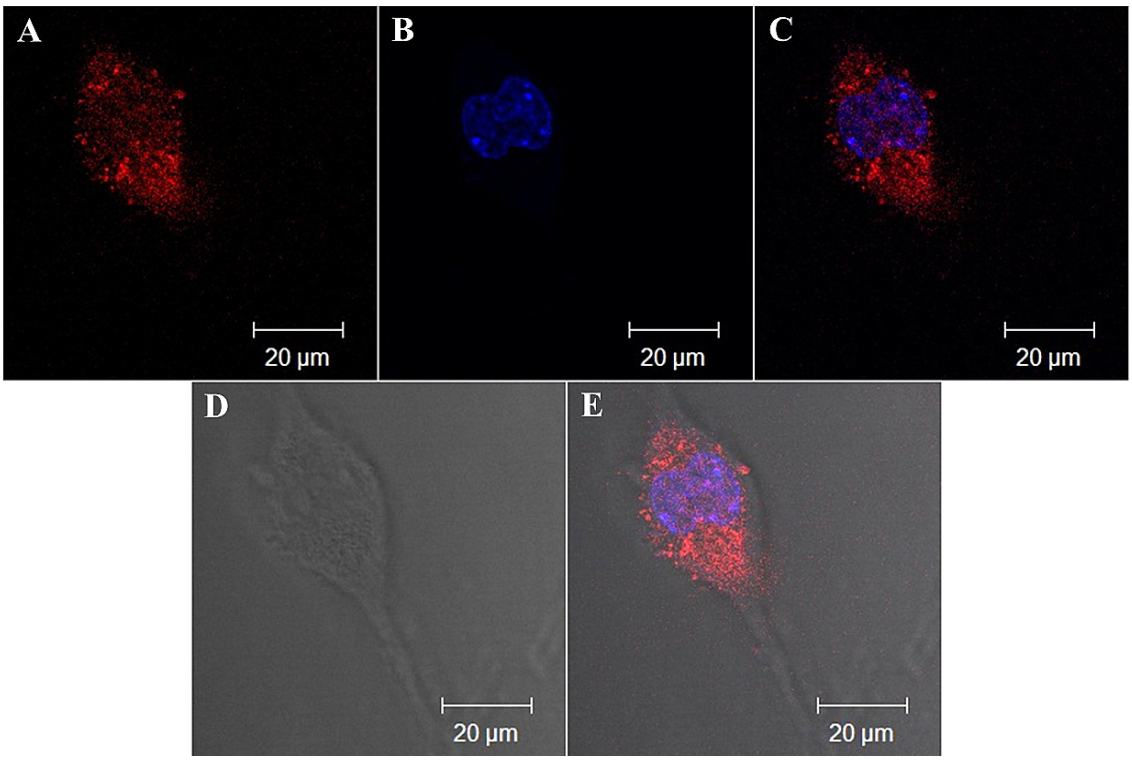

Figure 4. Example micrographs of an NG108-15 neuronal cell cultured with rhodamine B-labelled Au NRs (in red). Cells were cultured for 5 days in with Au NRs and stained for DAPI (blue). A) Clusters of RhB-labelled Au NRs uniformly dispersed inside the cytoplasm. B) Cell nucleus in the process of division. C) Merging of A and B showing the exclusion of Au NRs from the cell nucleus. D) Differential interference contrast image showing the natural cell morphology. E) Merging of C and D. Scale bars are 20 μm (reproduced from [54], with permission from John Wiley and Sons, copyright 2013).

the endocytosis pathway [55]. Subsequent studies identified the clathrin or caveolae receptors as the sites responsible for the internalization mechanism [56] and that membrane tension also plays an important role in the process [57]. It was demonstrated that uptake is controlled by the shape, dimensions and external charge of the particles [55]. In NG108-15s, it has been observed that Au NRs were taken up after one day of incubation. The localization was predominantly observed in the cell cytoplasm, with little or no nuclear or neurite internalization (Figure 3.2) [54]. In future work it would be of interest to investigate particle functionalizations with an affinity for the axons, which are the target in many INS applications. In that case the stability of the functionalized particles will also need to be considered.

Cells respond to external stimuli by activating different cellular pathways and therefore expressing specific sets of genes. Thus, transcriptional regulation can determine growth stage, differentiation status, development, fate and pathological status of cells. When NPs are used at high concentrations, they are likely to cause cell oxidative stress, production of reactive oxygen species (ROS), inflammation, cellular damage

or apoptosis *in vitro* [58, 59]. Therefore it is important to reduce the inflammatory responses by optimizing the NP concentration in tissues with further *in vivo* studies. Chiara to modify this after Jiawey fix the concentration values. Eom *et al.* found that the amplitude of compound nerve action potentials was increased when 3.4×10^9 nanorods were distributed in the vicinity of the plasma membrane of a rat sciatic nerve and exposed to laser irradiation [60]. Although histological analysis showed no tissue damage after an irradiation dose of 0.96 J/cm^2, more in depth studies on cellular pathway activations are required.

In NG108-15s, it has been shown that oxidative stress (measured with ROS production and nuclear factor kappa B (NF-κB) activation) was induced one hour after Au NR incubation [61]. This inflammation effect diminished over time, with a significant decrease detected in overall activation five hours post incubation. Only minor additional effects were identified on ROS production and NF-κB activation after laser irradiation of the endocytosed particles. Given the interest in NP models for biological applications [31], these findings are encouraging and decrease the probability of severe cytotoxic effects arising due to excess ROS [59, 62]. Interestingly, ROS production and NF-κB activation were not specific to the different surface chemistries of the NRs [61].

4. Stimulation of Nanoparticles

When laser light interacts with plasmonic NPs, two main processes are likely to occur: absorption and scattering. If the optical illumination matches the plasmon resonance wavelength, the absorption process often prevails over the scattering and excites the free electrons in the surface. The energy is then rapidly transferred to the crystal lattice as heat due to collisions, whereupon it is dissipated into the surrounding environment [63]. This localized heating after high laser irradiance has mainly been used to induce cancer cell death [64] or to release genetic materials such as RNAi from the Au surface [65]. Recent studies have shown that a lower laser exposure can be used to induce specific cell responses in neuronal cells [54, 66, 37]. A stimulatory effect was observed after exposing Au NRs in NG108-15 neuronal cells with laser irradiance between 1.25 and 7.5 W $\cdot$ cm^{-2}, based on the maximum neurite length, the number of neurites per neuron and the percentage of neurons with neurites (see Figure 4). The greatest neurite length was achieved with an irradiation dose of 7.5 W $\cdot$ cm^{-2}, with an average neurite length almost 36% higher than the non-irradiated sample [54]. Although this behaviour was not linked to the NR surface chemistry, it should be noted that the silica-coating is likely to have the highest photothermal and chemical stability after prolonged laser irradiation [45, 44].

Subsequently, intracellular Ca^{2+} transients were recorded in NG108-15 cells after laser irradiation of Au NRs using a modulated binary signal with variable frequencies (0.5, 1 and 2 Hz) and pulse lengths (between 20-100 ms) [66]. It was hypothesized that the transient heating arising from excitation of the LSPR of the NRs could serve to *i*) generate changes in the membrane capacitance, which could in turn open some

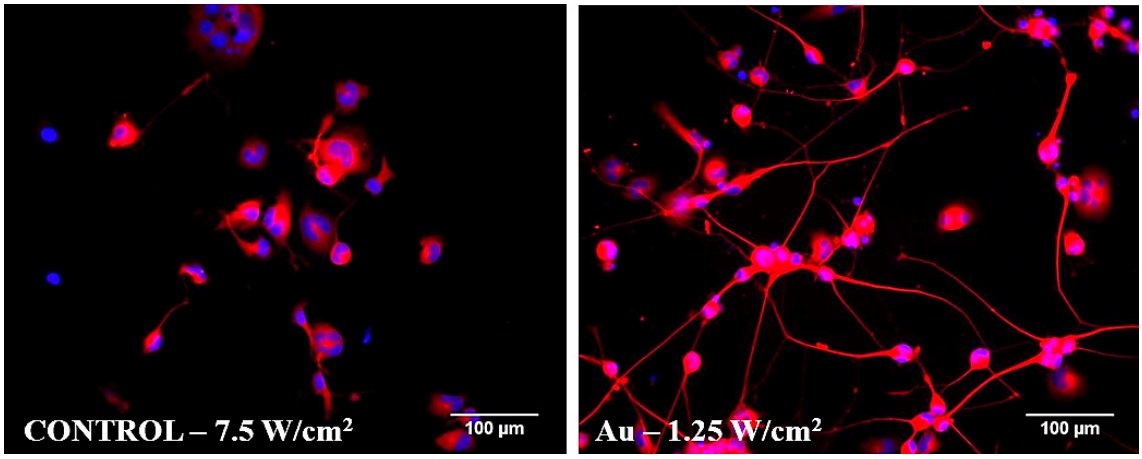

Figure 5. Examples of epifluorescence images of NG108-15 neuronal cells cultured alone (A) or with Au NRs (B) and exposed to different laser irradiances, as indicated in each panel. Samples were incubated for one day before laser irradiation. Cells were fixed and labelled for anti-β-III tubulin (red) and DAPI (blue) three days after laser irradiation. Scale bars are 100 μm (reproduced from [54], with permission from John Wiley and Sons, copyright 2013).

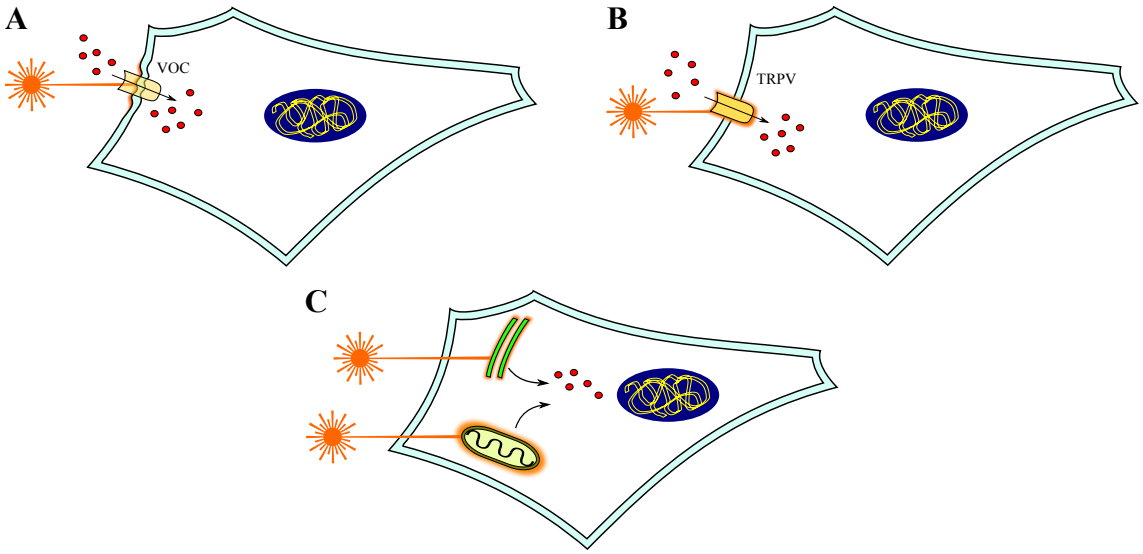

Figure 6. Three possible mechanisms for inducing Ca^{2+} waves by laser irradiation: (A) transient changes in the membrane capacitance which could in turn open voltage-operated channels (VOC), (B) activation of temperature-sensitive TRPV ion channels in the cell membrane, or (C) depletion of intracellular calcium storage organelles (e.g. endoplasmic reticulum and/or mitochondria).

voltage-operated channels [25], *ii*) activate temperature-sensitive ion channels in the cell membrane (e.g. transient receptor potential vanilloid (TRPV) channels) [26] and/or *iii*) deplete the intracellular calcium storage organelles (e.g. endoplasmic reticulum and/or mitochondria and/or Golgi apparatus). These mechanisms are illustrated in Figure 6.

In a more recent report, it has been observed that NIR laser illumination of Au NRs can be used to induce cell membrane depolarization and action potentials in primary

cultures of spiral ganglion neurons from early post-natal rats [37]. Whole-cell patch-clamping was used to simultaneously record the enhanced current and voltage activity in the neurons under laser exposure, following the procedure for *in vitro* investigation of INS outlined in [67]. Laser pulse lengths corresponding to energies as low as 5 μJ were able to evoke inward transmembrane currents (Figure 7A). The magnitude of the evoked current was proportional to the laser pulse length. For pulse lengths of 25 μs or longer, laser illumination was able to elicit action potentials in the neurons (Figure 7B).

As a comparison, neurons were incubated with Au nanospheres and tested using the same NIR laser. Spherical particles are relatively weak absorbers at the NIR laser wavelength. As a result, the laser-induced electrical activity of the neurons was much weaker than when Au NRs were used as absorbers. Without any NPs, patch-clamp recordings of the control neurons exhibit negligible electrical activity upon laser exposure. These findings demonstrated that direct stimulation from the NIR laser alone is unlikely to contribute to the observed electrical activity. Therefore the enhanced activity was attributed to photothermal stimulation, in which the interaction between the incident laser and Au NRs provided a thermal source in the vicinity of the neurons. This was confirmed by open patch pipette measurements of the temperature increase near the surface of the neurons containing nanorods [37]. Changes in the local temperature were in the range from 0.5°C to 6.0°C, showing a sub-linear dependence with the laser pulse length (or energy), as shown in Figure 7C. Temperature elevation profiles (inset in Figure 7C) were shown to change in accordance with the laser pulse lengths.

More recently, the first *in vivo* application of INS with irradiated Au NRs has been demonstrated by Eom *et al.*, using Au NRs of ∼80 nm in length with a laser wavelength of 980 nm [60]. The particles were continuously infused close to the plasma membrane of the rat sciatic nerve, whereupon laser irradiances between 0.32 and 2.23 J $\cdot$ cm^{-2} were applied. The laser exposure led to a six-fold increase in the amplitude of compound nerve action potentials and a three-fold decrease of the stimulation threshold. Histological analysis after the irradiation indicated that there was no evidence of thermal damage for doses lower than 1 J$\cdot$cm^{-2}. The enhanced response was attributed to local heating effects resulting from the excitation of the NR LSPR. The laser-induced CNAPs were inhibited in the presence of a voltage-gated Na^+ channel blocker (lidocaine), thus confirming that the response was due to neuronal activity.

5. Conclusion

The results reviewed in this paper have demonstrated the feasibility of using Au NRs as extrinsic absorbers to mediate the stimulation of neuronal cells with near infrared light. Although the details of conventional INS are not yet fully understood, the results for NR mediated stimulation so far appear to follow a very similar pattern. Significantly, while we have not been able to observe action potentials from INS on primary nerve cells *in vitro*, the cells were successfully activated by laser stimulation when coupled

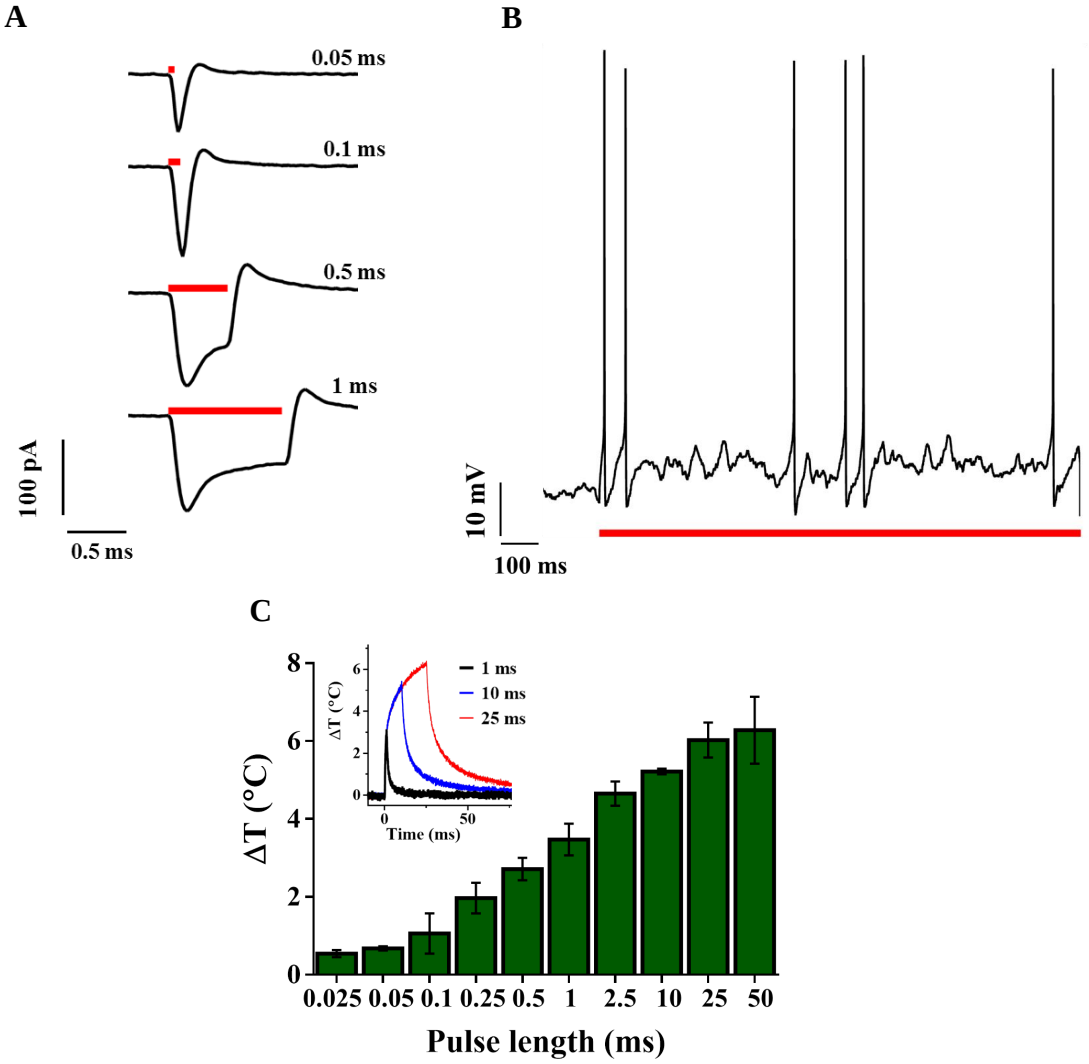

Figure 7. Gold nanorod-assisted photothermal stimulation of spiral ganglion neurons: (A) typical averaged voltage-clamp data of a neuron in response to laser pulses of different lengths; (B) current-clamp recording of a neuron showing action potentials fired in response to a single laser pulse lasting $\sim$ 1 s; (C) mean peak temperature change as a function of pulse length. Inset: representative temperature profiles for 1, 10 and 25 ms pulse lengths. Red bars indicate the laser timing and pulse lengths (reproduced from [37], with permission from John Wiley and Sons, copyright 2014).

with gold nanorods.

Although the irradiances reported in the results to date are similar to the levels reported for conventional INS, the actual laser power levels used are significantly lower, due to the more intense laser diode sources available in the near infrared. In effect, the source power can be focused more tightly, thus providing a clear advantage in situations where localized stimulation is required. It must also be noted that the power is primarily absorbed by the NRs, rather than by the ubiquitous water. Therefore

significant additional optimization of the process can be achieved by targeting the NRs to locations where the heating can be most effective e.g. the cell membrane. In addition to improved localization, the relatively fast thermal response of laser-exposed Au NRs may allow higher pulse repetition rates. Further modelling of the micron-scale transient heating associated with Au NRs may provide additional insights in this regard.

Despite the important potential advantages of Au NR absorbers for INS, this approach requires modification of the nerve tissue and thus loses the essential simplicity of conventional INS. However, the use of inert NPs deserves serious consideration, given the challenges associated with genetic modification required for optogenetic techniques. Similarly, the use of NP absorbers may be preferable to caged molecule approaches in that the NPs can in principle support high stimulation rates for extended periods of time. The demonstration of enhanced stimulation with continuously injected Au NRs by Eom *et al.* [60] suggests that this approach could be used to remotely manipulate and stimulate diverse excitable tissues. Nevertheless, it is clear that for Au - NR - enhanced INS to become a more broadly applicable technique, it is necessary to evaluate the long term biocompatibility of the particles. This question is linked to the need for stable targeting of the NRs to specific nerve cells or axonal structures, in order to capture the maximum benefits of spatially precise stimulation. Au NRs, with well established pathways for surface chemical functionalization, provide a promising platform to address this challenge in future [35, 50].

6. Acknowledgements

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