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Neural stimulation with optical radiation

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Abstract

This paper reviews the existing research on infrared neural stimulation, a means of artificially stimulating neurons that has been proposed as an alternative to electrical stimulation. Infrared neural stimulation (INS) is defined as the direct induction of an evoked potential in response to a transient targeted deposition of optical energy. The foremost advantage of using optical radiation for neural stimulation is its spatial resolution. Exogenously applied or trans-genetically synthesized fluorophores are not used to achieve stimulation. Here, current work on INS is presented for motor nerves, sensory nerves, central nervous system, and *in vitro* preparations. A discussion follows addressing the mechanism of INS and its potential use in neuroprostheses. A brief review of neural depolarization involving other optical methods is also presented. Topics covered include optical stimulation concurrent with electrical stimulation, optical stimulation using exogenous fluorophores, and optical stimulation by transgenic induction of light-gated ion channels.

Keywords

Cochlea; cochlear implants; deafening; electrical stimulation; infrared neural stimulation; laser; neuroprosthesis; optical stimulation; spatial selectivity

1. Introduction

Pulsed mid-infrared lasers have been suggested as a method for neural stimulation [1–3]. The use of lasers has several appealing features when compared to electrical stimulation: no direct contact is necessary between the stimulating source and the tissue, fine spatial resolution of stimulation can be achieved, no stimulation artifact is generated to deter simultaneous recording of electrical responses from the neurons, and there is no electrochemical junction between the stimulation source and the tissue. Furthermore, at many of the radiation wavelengths used for infrared neural stimulation (INS), the radiation can be easily coupled to an optical fiber. Limitations of INS relate to the laser–tissue interaction. Irradiation of tissue with infrared wavelengths will result in a transient temperature increase, which can cause thermal tissue damage. Although it was possible to stimulate auditory neurons through thin bony structures in the cochlea, thick absorbing or scattering layers above the target structure may prevent neural stimulation.

At present, only a small portion of the available optical parameter space has been investigated for INS *in vivo* [3–5]. Parameters that are optimized for one application, such as motor nerve stimulation, are not necessarily the ideal parameters for stimulation of a sensory system, such as the cochlea. Little research has been conducted *in vitro*, leaving a large gap in our understanding of INS. Selection of laser parameters, such as wavelength that dictates radiation scatter and absorption in the tissue, will significantly affect the success of stimulation. With further refinement and extension of the technique, INS has the possibility to become a useful tool in basic science research, as well as in clinical applications.

2. Optical stimulation of neurons

Research on stimulating neurons with visible light was reported as early as 1891 [6]. Subsequent experiments by Arvanitaki and Chalazonitis [7] demonstrated that inhibition and excitation can be triggered in nerve cells by irradiation with different wavelengths, ranging from the visible to the near-infrared. However, it is difficult to view these results as a logical progression towards the present method of neural stimulation. Most notably, the optical parameters used in early experiments differ significantly from those used more recently. Radiation wavelength, irradiation time, energy, as well as the optical properties of the irradiated tissue determine the laser–tissue interaction. Careful selection of optical parameters is crucial for INS. There are several categories into which previous studies can be grouped: (1) optical stimulation concurrent with electrical stimulation, (2) optical stimulation evoking a photochemical reaction through either an endogenous or exogenous chromophore, and (3) optical stimulation by other means.

For optical stimulation concurrent with electrical stimulation, a variety of optical sources, both continuous and pulsed, have been used to modulate electrically evoked neural activity. Booth et al. [8] studied the effect of continuous ultraviolet radiation ($\lambda < 370$ nm; cw irradiation for >2 min) on the excitability of myelinated nerve fibers. Irradiation of the node of Ranvier increased the electrical stimulation threshold and decreased the electrically evoked action potential amplitude. However, irradiation of the nerve's internodal region lowered the electrical stimulation threshold. Since irradiation changed neural responses only for radiation wavelengths below 320 nm, the authors concluded that a photochemical effect

on an unknown membrane substance was responsible for the results. Similar results were reported by Cook [9]. More recently, Olson et al. [10, 11] irradiated rat cerebellar tissue with a ruby laser ($\lambda = 694.3$ nm, $\tau_p = 100$ μ s, $Q = 0$ – 390 μ J, laser spot radius ~ 0.2 mm) or a dye laser (λ discrete bands between 490 and 685 nm, $\tau_p = 1$ μ s, $Q = 15$ – 360 μ J, laser spot radius ~ 3.5 μ m) simultaneously with electric current pulses. At low energies, irradiation had no effect on the electrically evoked neural function. However, at higher energies, laser irradiation reduced the electrical excitability of the neurons. At energies where laser irradiation modified electrically evoked neural activity, mitochondrial damage was seen in electron micrographs. The authors excluded the possibility of a photochemical reaction and concluded that the irradiation induced a local heating in the mitochondria, which led to decreased neural activity and to tissue damage [10, 11]. Uzdensky and Savransky [12] demonstrated an initial increase, followed by a decrease, in the frequency of electrically evoked action potentials when irradiating neurons with a dye laser ($\lambda = 434$ – 600 nm, $\tau_p = 15$ ns, $f = 400$ Hz, $E = 0.25$ – 5 kW/cm²). They found a maximum change in the electrically evoked neural responses with 460-nm radiation. Using the same power density, they did not observe any change in electrically evoked neural activity with 580-, 600-, or 632-nm radiation. The authors hypothesized that irradiation effects were a result of light absorption by flavins and a subsequent calcium (Ca²⁺) release from mitochondria. Wesselmann et al. [13] utilized a pulsed, Nd:YAG laser ($\lambda = 1064$ nm, $\tau_p = 8$ ns, $f = 10$ Hz, $E = 1.4$ – 145 W/cm²) to modify the electrically evoked activity from spinal cords as well as dorsal roots and peripheral nerves [14]. In both studies, the compound action potential (CAP) amplitudes decreased, while the tissue temperature increased during irradiation, up to 60 °C [15]. Another group investigated the effects of Nd:YAG irradiation ($\lambda = 1064$ nm, $\tau_p = 150$ μ s, $f = 10$ – 20 Hz, $Q = 30$ – 150 μ J) on excised spinal nerves. They described a decrease in electrically evoked CAP amplitudes that was dependent on the radiation dose [16]. The amplitude of the electrically evoked CAP was less likely to return to pre-irradiated levels with increasing laser energy. The authors suggested that laser irradiation blocked the action potential (AP) propagation through the irradiated area of the nerve. The CAP latency decreased during irradiation, which was likely caused by an increase in tissue temperature. Bagis and coworkers [17–19] investigated the effect of a pulsed gallium-arsenide laser ($\lambda = 904$ nm, $\tau_p = 220$ ns, $f = 4$ – 128 Hz, $H = 0.005$ – 2.5 J/cm²) on frog gastrocnemius muscles and sciatic nerves and found no influence of laser irradiation on measurements, such as electrical stimulation threshold, action potential latency, or duration.

Neural stimulation may also occur via a photochemical reaction, which is initiated by the absorption of the radiation energy. Primary photochemical processes occur as an immediate result, however, secondary chemical processes may follow. In most cases, a photochemical reaction is achieved by the addition of an exogenous or synthetic chromophore. Early work in photochemical neural stimulation was achieved by Farber and Grinvald [20] to map out synaptic connections of leech neurons. They synthesized a photosensitive molecule that bound to the nerve membrane, which was then irradiated with a HeNe laser ($\lambda = 632.8$ nm, $\tau_p =$ up to 5 s, $P = 11$ mW) to depolarize the neuron. Their results suggested that laser stimulation occurred via transient, light-induced channel activation that was not ion specific [20]. The photostimulation often caused irreversible photochemical damage to the nerves. Hirase et al. [21] imaged cortical neurons with a two-photon fluorescence microscope. Upon irradiation with a pulsed Ti:sapphire laser ($\lambda = 790$ – 850 nm, $\tau_p = 130$ fs), APs could be triggered without an exogenous fluorophore. In their experiments, they observed two regimes of optical stimulation. Type 1 stimulation occurred at laser powers of 200–400 mW. The underlying mechanism likely involved a two-photon reaction with endogenous chromophores. When an exogenous fluorophore was added, they could not stimulate the neural tissue with wavelengths longer than 800 nm, whereas stimulation always occurred when irradiating with wavelengths 790 nm or shorter. Type 2 stimulation was achieved at higher laser energies. The authors hypothesized that the second type of stimulation resulted

from microholes in the cell membrane, which allowed ion flux into the cell and subsequent neural depolarization.

Photochemical neural activation can also involve the addition of a “caged” molecule that is released upon irradiation. Caged molecules are molecules that were rendered inert by chemically modifying the structure of a bioactive molecule. Irradiation transforms and/or cleaves the caged molecule to restore the biological activity, which is commonly referred to as “photorelease” or “uncaging” [22]. The resulting active molecules can be agonists or antagonists. Thus, photolysis of caged compounds is a method for using light to switch biological processes on or off. In 1990, Wilcox et al. [23] reported the synthesis of light-sensitive precursors to neurotransmitters. Dalva and Katz [24, 25] used this technique to map the developing synaptic connections in the primary visual cortex of ferrets by uncaging photolabile glutamate. Since then, many investigators have taken advantage of these photolabile neurotransmitter precursors, most notable glutamate, in their research (for reviews, see Callaway and Yuste [26], and Eder et al. [27]).

Another technique for photochemical neural activation in mammalian neurons is the expression of channel-rhodopsins in the cell membrane of the neuron. Channel-rhodopsins are naturally occurring, rapidly gated, light-sensitive algal proteins [28–33]. Most often, neurons are transfected with channelrhodopsin-2 (ChR2), after which the cells can be depolarized upon irradiation with 500 nm (blue) light pulses [31, 34]. Results have been demonstrated in several animal models, including mice and monkeys [34–36]. The research field, termed photostimulation or “optogenetics”, is rapidly expanding and other chromophores have been identified. The expression of halorhodopsin (Halo/NpHR), for example, can be used to inactivate neurons when they were irradiated with yellow (577 nm) light [37, 38]. Readers are referred to a recent review by Kramer et al. [39].

Through endogenous chromophores, photochemical reactions and neural stimulation can be initiated. Early studies were carried out by Arvanitaki and Chalazonitis [7] and Fork [40]. Each of these researchers irradiated *in vitro* neural preparations with continuous or quasi-continuous light and measured the rate of action potentials. Fork irradiated the abdominal ganglion of *Aplysia californica* for 2–5 s, using continuous blue (488 nm), green (515 nm), or near-infrared radiation (1060 nm) [40]. The objective of the study was to map cellular interconnections of *Aplysia* neurons. Stimulation of the neurons was possible without damaging the cells. Fork examined possible mechanisms for the light-evoked neural stimulation by changing the ion content of the bathing solution and by applying different ion channel blockers. He concluded that irradiation opened sodium-ion channels that may have activated the sodium–potassium ion exchange pump. However, the persistence of residual light-evoked responses in the presence of ouabain suggests a more complex mechanism. Arvanitaki and Chalazonitis [7] used several preparations, such as *Aplysia* and a stained *Sepia* giant axon, for optical neural stimulation. They stimulated with visible (400–700 nm) and infrared (750–4000 nm) radiation. In response to the irradiation, most neurons were depolarized. However, some nerve cells in an *Aplysia* preparation decreased their neural activity during infrared irradiation. The hypothesized mechanism for both excitation and inhibition was the absorption of light by an undetermined cellular molecule.

Balaban et al. [41] used a HeNe laser ($\lambda = 632.8$ nm, $E = 0.05$ to 4 W/cm², cw) to irradiate snail ganglia. They were able to modulate the rate of APs in spontaneously active neurons, but could not evoke APs in silent neurons. The authors concluded that the temperature effects of irradiation were responsible for the results. A recent study has shown that irradiation with a pulsed, near-infrared diode laser ($\lambda = 980$ nm, $\tau_p = 4$ ms to 400 ms) induced inward currents in dissociated cell cultures of nociceptive rat dorsal root ganglion cells [42]. At the stimulation threshold of 2.8 J/cm², they calculated a temperature of 42 °C

at the neurons. Interestingly, the radiation-induced inward current decreased in amplitude with repeated stimulation. The authors suggested that the primary mechanism of stimulation was a thermal effect from the laser. Furthermore, they demonstrated that all of the laser-stimulated neurons were sensitive to capsaicin, which binds to a temperature-sensitive ion channel.

3. Neural stimulation with a pulsed infrared laser

3.1. Stimulation of peripheral nerves

Sciatic nerve—Recently, Wells et al. [1,4] have used a pulsed infrared laser to stimulate the sciatic nerve. This method of stimulation has been termed infrared neural stimulation, or INS. It is defined as the direct induction of an evoked potential in response to a transient, targeted deposition of optical energy. The foremost advantage of using pulsed infrared radiation for neural stimulation is its spatial resolution. Only neural tissue that is directly in the optical path will be stimulated.

Several stimulation sources have been used to stimulate the sciatic nerve at low energies, including a free-electron laser (FEL, $\lambda = 2000\text{--}6000\text{ nm}$; $\tau_p = 5\text{ }\mu\text{s}$), a Ho:YAG laser ($\lambda = 2120\text{ nm}$; $\tau_p = 350\text{ }\mu\text{s}$), or a pulsed diode laser ($\lambda = 1860\text{ nm}$; $\tau_p = 10\text{ }\mu\text{s}$ to 10 ms). Action potentials measured from nerve bundles show similar waveforms when stimulated electrically or optically. Neural stimulation was possible at optical wavelengths that are moderately absorbed in neural tissue (Fig. 1). The estimated optical penetration depth in neural tissue for these wavelengths is 200 to $1500\text{ }\mu\text{m}$ [43, 44], assuming primarily water absorption [45,46]. For INS, exogenously applied or transgenically synthesized fluorophores are not used to achieve stimulation. Wells et al. found that irradiation of different portions of the sciatic nerve elicited contraction in different muscle groups. Also, the authors measured the wavelength variability of the neural stimulation. When examining the stimulation and ablation thresholds, the ablation threshold scaled strongly with the wavelength-dependent water absorption coefficient, as expected (Fig. 1) [4, 47]. However, the stimulation threshold scaled less strongly. Wells et al. found that the safety ratio of stimulation threshold to ablation threshold followed the water absorption curve, with the best safety ratio measured around 2120 nm .

One recent paper showed that the threshold for optically stimulating the sciatic nerve could be reduced with the simultaneous presentation of a subthreshold electrical stimulus [48]. Reducing the energy required for INS would, thereby, increase the safety margin between threshold and tissue damage. Other researchers have investigated alternative wavelengths for infrared nerve stimulation, namely in the $1400\text{--}1600\text{ nm}$ range [49]. Initial reports indicated a depolarization of the sciatic nerve from exposure to 1450-nm , 1495-nm , or 1540-nm radiation. However, the pulse durations at which depolarization was shown were significantly longer ($\sim 100\text{ ms}$) and the fluences were higher than those demonstrated in earlier INS experiments.

To explore whether INS irradiation of the nerve resulted in tissue damage, Wells et al. stimulated rat sciatic nerves with a Ho:YAG laser [50]. Nerves were removed either immediately or two weeks poststimulation. The nerves were assessed histologically for thermal damage. Laser parameters studied included upper limits for radiant exposure, repetition rate, and duration of stimulation. Radiant exposures with $<1\%$ probability of thermal tissue damage ($0.66\text{--}0.70\text{ J/cm}^2$) are significantly greater than radiant exposures required for reliable stimulation ($0.34\text{--}0.48\text{ J/cm}^2$). The upper limit for the repetition rate that ensures nondamaging laser stimulation in peripheral nerves occurred near 5 Hz . The maximum duration for constant low repetition rate stimulation (2 Hz) was approximately 4

min with adequate tissue hydration [50]. Note, these results differ from the findings for the auditory nerve (see below for details).

Nervus pudendus—In another series of experiments, rat cavernous nerves were stimulated with a pulsed laser in the near-infrared [51, 52]. A thulium fiber laser ($\lambda = 1870$ nm; $\tau_p = 2.5$ ms; $f = 10$ Hz) was used to stimulate the nerves with 7.5 mJ/pulse. During the experiments, the nerves were stimulated for 60 s periods of time. A significant increase in the intracavernosal pressure was detected during laser stimulation, with the pressure returning to baseline values after stimulation.

3.2. Stimulation of cranial nerves

Facial nerve—Based on the experiments by Wells et al., infrared radiation was used to stimulate the facial nerve [2]. Gerbil facial nerves were exposed to 250 μ s pulses of 2120 nm radiation at a repetition rate of 2 Hz and were delivered via a 600- μ m diameter optical fiber. During the stimulation, muscle action potentials were recorded. Fig. 2 shows compound muscle action potentials (CmAPs) evoked with either optical radiation or electrical current. Qualitatively, the CmAPs were similar in shape. However, the onset of the optically evoked CmAP was delayed relative to the electrical CmAP. Radiant exposures ranged between 0.71–1.77 J/cm² to evoke CmAPs, which were simultaneously measured at the facial muscles (m. orbicularis oculi, m. levator nasolabialis, and m. orbicularis oris). To determine acute damage thresholds, nerve samples were examined. Histology showed tissue damage at radiant exposures of 2.2 J/cm², but no apparent damage at radiant exposures of 2.0 J/cm².

Again, the spatial selectivity of infrared nerve stimulation has been demonstrated in these experiments at the facial nerve trunk. Different nerve bundles within the main nerve trunk were individually stimulated when the radial location of the stimulation site was varied (Fig. 3). Similar spatial selectivity has been reported by Wells et al. for the rat sciatic nerve [1] where the authors were able to individually stimulate single muscles (e.g.: the hamstring muscle) while scanning the small diameter optical fiber over the sciatic nerve. In contrast to INS, bipolar electric stimulation evoked strong and unselective responses in all muscle groups innervated by the sciatic nerve.

Auditory nerve—One of the advantages of INS versus the use of electric current is the possible simultaneous but nonoverlapping stimulation of many discrete sites along the cochlea. Activation of small neuron populations would overcome some of the issues of contemporary cochlear prostheses, which use electrical current to stimulate the neurons. The current spreads in the cochlea and selective stimulation of the neurons is difficult [53–58]. At present, INS of the auditory nerve has been studied in the gerbil, the mouse, the guinea pig and the cat.

In the first series of experiments, a Ho:YAG laser ($\lambda = 2120$ nm; $\tau_p = 250$ μ s; $f = 2$ Hz) was used to stimulate the gerbil auditory nerve [3]. The laser was coupled to a 200- μ m diameter optical fiber, which was placed in front of the cochlear round window. The optical beam was directed towards the spiral ganglion cells. Recordings of the cochlear neural responses demonstrated that optical stimulation is possible. CAPs were evoked directly in response to the laser pulses. An increase in radiation energy induced a monotonic increase in the evoked responses. Subsequent experiments with different diode lasers ($\lambda = 1844$ –1940 nm; $\tau_p = 5$ μ s–1 ms; $f = 2$ –1000 Hz) showed that the patterns of evoked CAPs changed with increasing pulse duration (Fig. 4) [5, 59]. The variability in the input–output curves was, in part, due to differences in the physiological state of each animal and differences in optical fiber

placement. In acutely and chronically deaf gerbils, thresholds for optically evoked CAPs were not significantly elevated when stimulating with short pulse durations [60].

Long-term acute stimulation was achieved for 6 h in the gerbil at 200 Hz stimulation rate, with stable CAP amplitudes for the duration of the stimulation. Similar results have been shown in the cat (unpublished). CAPs were evoked with each pulse when stimulating at 200 Hz. However, single auditory nerve recordings from low spontaneous rate neurons show, on average, a firing efficiency of ~ 40% at a 200-Hz laser stimulation rate [61].

Transiently expressed transcription factor, c-FOS, was expressed in activated neurons to identify the spatial area of the cochlea that was stimulated. Immunohistochemical staining for c-FOS in the cochlea showed a small area of optical stimulation, which occurred directly opposite to the optical fiber [5]. Additionally, masking data and inferior colliculus measurements (Fig. 5) indicated that the laser could stimulate a small population of cells similar to an acoustic toneburst. The top two graphs in Fig. 5 show the spatial extent of cochlear optical stimulation as measured from a multichannel, penetrating electrode in the guinea pig inferior colliculus. The tonotopic location of the electrode contacts was first mapped using acoustic tones. Next, the cochlea was acutely deafened and an optical fiber was inserted through a cochleostomy in the basal turn. Neural responses to the laser pulses were recorded and ROC (receiver operating characteristics) analysis was applied to calculate d' values as follows:

$$d' = \frac{\mu_2 - \mu_1}{\sigma},$$

where μ_1 and μ_2 are means of normally distributed data for two optical energy levels and σ^2 is the common variance. The d' (discriminability) quantifies the separation between the two distributions. For example, $d' = 0$ between two energy levels would suggest that the neural responses evoked by optical stimulation at the two levels are indistinguishable. $d' > 1$ suggests that the distributions are more separate, i. e. stimulation at the higher energy level resulted in more neural responses. The data in Fig. 5 show that optical stimulation of the cochlea could achieve selective stimulation, similar to the extent of acoustic tones. In addition, by moving the optical fiber, the spatial location of stimulation in the cochlea (correlated to the acoustic frequency by the tonotopic map) could be modified.

Vestibular nerve—Vestibular prostheses, based on electrical stimulation, are currently being studied as a means to reduce imbalance and disorientation caused by vestibular dysfunction. However, electrically based vestibular prostheses suffer from current spread, which can lead to stimulation of unintended semicircular canal(s) and subsequently encode incorrect sensory information. Optical stimulation of the vestibular system has been investigated as a means to overcome the spread of electric current. Harris and coworkers [62] studied whether infrared radiation could be used to evoke neural activities from the vestibular nerve. Optical radiation at 1840 nm wavelength ($\tau_p = 10 \mu\text{s}$ –1 ms) was effective at evoking neural responses from the eighth nerve at a location where vestibular and auditory fibers were present. Power levels as low as 200mW evoked a response. Direct irradiation of the ampullae produced an evoked potential at the maximum radiation energy obtained from the diode laser (Fig. 6). However, optical stimulation of the ampullae did not evoke detectable eye movements.

In addition to the eighth nerve, they also stimulated the facial nerve. Careful inspection of the optically evoked facial nerve CAPs reveals a component at the onset of the optical pulse and at the offset of the pulse (data not shown here) [62]. The two CAP components became obvious with increasing time between the pulse onset and offset (longer pulse durations).

Although offset responses have not been reported by others for the facial nerve or the sciatic nerve, they could be seen in some auditory neural responses. Experiments have yet to be conducted to determine the source of the offset response.

In a different set of experiments, Bradley stimulated Scarpa's ganglion in gerbils with diode lasers ($\lambda = 1844\text{--}1877\text{ nm}$; $\tau_p = 5\text{ }\mu\text{s--}1\text{ ms}$; $f = 2\text{--}1000\text{ Hz}$), while recording activities from single vestibular nerve fibers. The experiments showed an evoked CAP but did not result in detectable changes of single-fiber activities.

Recently, it has been shown that INS can activate vestibular hair cells [63, 64]. In experiments conducted in toadfish, *Opsanus tau*, stimulation was directed towards the neuroepithelium housing the sensory hair cells. Measurements from horizontal semicircular canal afferents showed a mix of inhibitory and excitatory responses evoked by INS. A small subset of afferents displayed a mixed response, comprised of an onset inhibitory response followed by an excitatory phase with tonic stimulation. The time course for the excitatory phase of the mixed units was similar to that of the excitatory afferents. The authors hypothesized that the excitatory vs. inhibitory afferent responses may correlate with the dynamic adaptive properties of afferent responses observed during mechanical stimulation [65]. Primary semicircular canal afferents in the toadfish are known to receive convergent inhibitory (GABA) and excitatory (glutamate) synaptic input from hair cells that ultimately shape the afferent discharge response [66, 67]. Data from INS experiments in the vestibular system indicated that afferents receiving glutamatergic inputs may increase their discharge rate with infrared stimulation. This is consistent with depolarization of hair cells and increased tonic release of glutamate. On the other hand, afferents that synapse on combinations of glutamatergic and GABAergic hair cells were observed to reduce their discharge rate with INS.

Despite the fact that studies showed vestibular neural responses to INS, no study has yet demonstrated that the stimulation is selective. It may be that the Crista ampullaris, the sensory organ of the vestibular system, is insensitive to optical stimuli and/or that optical radiation stimulates remote afferent dendrites of the vestibular nerve. It is unknown if selective stimulation of afferent nerve fibers from individual canals can be achieved once they have collected in the nerve trunk.

3.3. Stimulation of the central auditory system in vivo

Lee et al. [68] have demonstrated that neurons of the central auditory system can be stimulated via INS. In acute animal experiments, a 400- μm diameter optical fiber was placed on the surface of the cochlear nucleus to irradiate the tissue with diode lasers ($\lambda = 1849\text{--}1865\text{ nm}$; $\tau_p = 5\text{ }\mu\text{s--}10\text{ ms}$; $f = 2\text{--}1000\text{ Hz}$). An optically evoked auditory brain-stem response (oABR) was recorded and resembled a multi-peaked ABR evoked by acoustic stimulation. The oABR waveforms had latencies between 3 and 8 ms, longer than ABRs evoked by direct electrical stimulation of the same region. Reproducible oABRs were evoked at thresholds as low as 169 mJ/cm², with 50 μs pulse duration and 5 Hz repetition rate. The oABR was stable during continuous optical stimulation for thirty minutes. Control experiments, in which the optical path was blocked and the rat was euthanized, eliminated the oABR. No thermal tissue damage was found on histological examination when stimulating with pulse durations less than 1 ms and radiant exposures less than 2.05 J/cm².

3.4. Stimulation of in vitro preparations

Recently, Rhee et al. [69] stimulated cultured rat sensory neurons with a near-infrared pulsed laser. They have used primary sensory neurons from the inferior ganglia of rat vagal nerves. In their experiments, they coupled the output of an 1850-nm diode laser to a 200- μm

silica fiber and were able to stimulate the neurons. These neurons express the vanilloid transient receptor ion channel (TRPV1), which is a nonselective cation channel that opens in response to temperature changes. The channels have an approximate 10:1 higher affinity to calcium than to sodium. To monitor neural excitation, the authors have, therefore, monitored the intracellular calcium concentration using the fluorescent calcium indicator fura-2. Brief trains of 2-ms IR pulses (~ 5 W instantaneous power) activated the TRPV1 ion channels rapidly and reversibly. Identical INS did not increase intracellular calcium in the absence of extracellular calcium, or after the application of a TRPV1 channel blocker.

Cayce et al. used the FEL ($\lambda = 2150\text{--}5300$ nm; $\tau_p = 5$ μ s; $f = 6\text{--}30$ Hz) to stimulate thalamocortical slices from the rat [70]. They demonstrated that higher repetition rates required lower energy to stimulate the neurons.

Other researchers working towards an all-optical stimulation and recording system have recently used *in vitro* preparations of lobster nerves and optically stimulated the nerves with 0.4–2-ms long pulses at 1850 nm [71]. The authors noted that longer pulse durations allowed only 2–4 repetitions of laser irradiation before nerve damage occurred. Readers should take caution in directly comparing these data with previous data because the energy output of the optical fiber and the spot size on the tissue were unknown.

4. Possible mechanisms for optical stimulation of neurons

Although some regimes of optical stimulation occur via photochemical interaction (see earlier sections), it is unlikely that pulsed, mid-infrared lasers, such as the FEL, Ho:YAG, or Aculight diode lasers, evoke neural responses via a photochemical reaction. The individual photon energies emitted by these lasers are significantly lower than the energies required to move an electron to an excited state, as is needed for a photochemical reaction. For example, the energy in individual photons from the Ho:YAG is ~ 0.58 eV. The value corresponds to a ~ 52 kJ/mol bond energy. Typical ion bond energies are larger and are in the range of 100–1000 kJ/mol. In addition, data from Wells et al. [1] did not identify a particular wavelength between 2000 and 10 000 nm at which the laser stimulation was significantly enhanced, which would have suggested a photochemical reaction.

The energy required for a primary photomechanical mechanism is much higher than used by Wells et al. and Izzo et al., so it is unlikely that this would be the primary mechanism of pulsed, mid-infrared laser stimulation of nerves [1, 3–5]. Moreover, the parameter space of INS is outside of stress confinement. Stress confinement occurs when the optical energy accumulates in the tissue before a laser-induced stress wave can propagate out of the irradiated area, leading to large stress waves [72]. For stress confinement to occur when irradiating with these wavelengths, the pulse duration should be shorter than 500 ns, which is orders of magnitude shorter than the pulse durations used for INS.

For photomechanical effects, both laser-induced stress waves and volumetric thermal expansion were considered as a mechanism for the stimulation. Wells et al. [73] argued against a photomechanical mechanism from stress-wave generation, because the stimulation threshold of the sciatic nerve was independent of laser pulse duration (all tested at a nearly constant wavelength or penetration depth). Furthermore, all pulse durations tested evoked an action potential. When varying the pulse duration, the experimental results for the peripheral nerve and the auditory system differ. For the deafened cochlea, shorter pulse durations are apparently more efficient in evoking an action potential. This conclusion was reached based on graphs showing the peak-to-peak amplitude of the action potential versus the energy. However, if the same CAP amplitudes were plotted versus the radiation power, shorter pulse durations no longer appeared more efficient (Fig. 7). In other words, the results suggest that the transient of the optical pulse was the important factor for cochlear INS and not the total

energy. Although it appears that there are differences between motor nerve and cochlear nerve INS results, one has to understand that the criterion to determine threshold differed. While in the sciatic nerve experiments a visual muscle twitch was the threshold criterion, for the cochlear experiments a selected value for the CAP amplitude served as threshold. Muscle twitch requires functional recruitment of a large number of fascicles, whereas small CAP amplitudes may reflect the depolarization of only a small number of auditory nerve fibers.

Wells et al. [74] concluded that INS occurred via a photothermal mechanism and measured the temperature rise associated with stimulation of the sciatic nerve. The radiation is likely absorbed by water in the tissue and, subsequently, transferred to heat. Wells et al. also concluded that a temporal and spatial thermal confinement is necessary to achieve infrared neural stimulation. Thermal confinement exists when the thermalized optical energy delivered by a single pulse accumulates in the irradiated tissue before any of the heat can dissipate through conduction or convection. For thermal confinement to be achieved at these wavelengths, the optical pulse duration is typically longer than ~ 500 ns and shorter than ~ 200 ms.

It is currently unknown how a transient temperature rise induces neural depolarization. Potential mechanisms include thermal activation of an ion channel, thermally induced biophysical changes in the membrane (increased ion-channel conductance, a decrease in Nernst equilibrium potential), formation of a membrane pore, or a general expansion of the neural membrane that facilitates the flux of ions. Researchers have used laser-induced stress waves to increase the permeability of the cell membrane for molecular delivery [75, 76]. Laser-assisted molecular delivery uses laser parameters for stress confinement to achieve reliable permeabilization, while preserving the cellular viability. The photomechanical effects of INS from volumetric expansion could not be altogether excluded since a small, but not negligible, displacement of tissue did occur at radiant exposures slightly above stimulation threshold. Wells et al. measured an expansion of the sciatic nerve membrane of approximately 300 nm within 350 μ s at stimulation threshold [74]. Although generation of a pressure wave through volumetric thermal expansion is possible in thermal confinement, there is no direct evidence yet that volumetric thermal expansion depolarizes the neurons [60, 74].

An alternative mechanism for INS could be the direct activation of heat-sensitive ion channels, which are present in nerves. The channels are termed the transient receptor potential (vanilloid) or TRPV channels [77–80]. TRPV1 is most well known for being activated by the chemical capsaicin, the main ingredient in hot chili peppers that produces a burning sensation. TRPV1 channels are also opened in the presence of other vanilloid compounds, acid (pH ~ 5.9), and heat ($\sim 43^\circ\text{C}$), making it a key channel in peripheral nociception. TRPV channels are found in small neurons, including the sciatic nerve, the dorsal root and trigeminal ganglia of rats [80], as well as cochlear structures of both the rat and guinea pig [81–83]. TRPV ion channels are expressed centrally at the cell body and in the neuron's periphery.

Experiments to study the mechanism of INS are underway. However, no candidate has emerged as the definitive cause for neural depolarization. For future studies, the thermal and mechanical aspects of the laser-deposited energy should be decoupled. In reality, however, this is a difficult task. Ideally, many mechanism studies could be carried out using neuronal cultures. When using infrared radiation to stimulate a cell culture, the surrounding water-based environment will absorb the energy and a transient temperature increase will occur, regardless of the presence of neurons and any neuronal stimulation. If the measurement method is susceptible to a temperature increase (e.g. metal electrode, patch pipette,

fluorescent dye, etc.), any neural responses may be confounded by a direct temperature effect on the measuring tool. Care should be taken in formulating INS mechanism studies, though the results will advance our understanding of INS.

5. Considerations for INS-based neural prosthesis

The goal for neuroprostheses is to restore neural function to the fidelity of a healthy system. Contemporary neural prostheses aim to achieve this via electrical stimulation of the remaining neurons. Despite the research advances and clinical implementation of neuroprostheses, there are several challenges that face neurostimulation (see Grill et al. [84] for a recent review of neuroprosthesis challenges). One of the main hurdles for neuroprostheses is achieving spatially and temporally selective stimulation. Several strategies have been employed to increase the spatial selectivity of electrical stimulation over a conventional monopolar stimulation paradigm. A multipolar electrode configuration has been employed to increase the selectivity of stimulation. However, more power is required to generate threshold responses [85, 86]. Cuff electrodes have been successfully used in several chronic animal and human studies to stimulate motor neurons, and the optic nerve [87–89]. However, drawbacks of this method can include nerve damage from the cuff [90, 91]. Penetrating electrodes also allow for more spatially focused stimulation by inserting the stimulating source into the nerve tissue. By compromising the structure and integrity of the nerve, penetrating electrodes can cause inflammation, edema and, subsequently, impair neural conduction [92, 93]. INS can confine the stimulated volume of tissue and may be another means of increasing spatial selectivity of neural stimulation. Another advantage of INS is the lack of an electrochemical junction. The fluid interface between the active electrode and the tissue, which is not needed for INS, is a common location for device failure [94].

However, before implementing an INS prosthesis, several design considerations must be made. In general, the stimulation source (optical fiber) must be in close proximity to the neurons of interest (see discussion in next section). While an optical-fiber-based prosthesis may be suitable for a low-density application (stimulation of 1–5 distinct sites), prostheses that encode more dense information, such as cochlear implants and visual prostheses, would be difficult to implement with 50–100 or more individually addressed optical fibers. Possible design solutions for these applications include a scanning optical source that sequentially addresses multiple stimulating sites or a high-density array of small solid-state optical sources that can simultaneously stimulate multiple neural populations. In addition to the type of source used, the placement of the optical source has implications for the prosthesis design. An external source would allow for a higher power, larger battery capacity, and easy modifications to the stimulating source. However, by transmitting the optical energy transcutaneously, a significant portion of the optical power would be lost. Percutaneous links, while possible, are less desirable due to the risk of infection. A fully implantable INS prosthesis is desirable because it eliminates the transcutaneous link except for battery charging. However, hardware upgrades are difficult with a fully implantable device. Furthermore, implanted optical sources need to be more efficient than externally mounted sources to reduce the heat generated by powering the device. It remains to be seen if a solid-state device can be constructed with the size and power requirements to be fully implanted and to stimulate neurons.

6. Limitations of INS

INS as a tool for neural depolarization is not a panacea. While the radiation energy can be delivered through air in a noncontact mode, water and tissue along the optical path will reduce the incident energy. For INS, typical wavelengths used were between 1840 and 2120

nm. Assuming that water is the sole absorber, the radiation wavelengths correspond to penetration depths of 100 to 1500 μm . In other words, the radiation energy at the tip of the optical fiber or at the radiation source decreases with each penetration length by 63%. Therefore, when implementing INS, one has to consider the distance and the types of materials between the radiation source and the target neuron. When the optical power density at the neuron to be stimulated is too small, membrane depolarization does not occur, and the neuron/axon is not activated. On the other hand, if the optical power density at the neuron is too large, then damage occurs. Since the thresholds between effective stimulation and damage for peripheral nerves are typically less than one order of magnitude, the amount of radiation energy delivered to the neuron is of great importance.

The above statements only take tissue optical absorption into account. The presence of highly scattering tissue, such as bone, in the optical path will increase the spot size and may decrease the selectivity of stimulation.

Since INS targets a restricted volume of tissue and the optical energy spreads very little in water, the placement of the stimulating source needs to be accurate relative to the neurons to evoke a response. While this feature is desirable for some applications, it will be a detriment to others. In addition, the extremely selective stimulation can make it difficult to detect the neural responses.

7. Conclusions and future directions

The experiments have demonstrated that optical radiation can be used to stimulate neurons. With the recent development of a compact pulsed infrared laser to evoke neural responses, it has been validated that stimulation with optical radiation is spatially selective. Stimulation with optical radiation has advantages over stimulation with electric current. The differences between stimulation modalities could be selectively exploited for the next generation of neural interfaces and as a neurophysiology research tool. INS, as a tool, could advance our understanding of many neuroscience questions and lead to significant findings that would otherwise not be possible with conventional stimulation paradigms. In addition, the possibility of using INS for a neural prosthesis has its appeal. Many steps are required between technological conception and maturity of an INS-based prosthesis. The steps include biological safety and compatibility, and engineering a practicable device.

With the objective to design and build INS prostheses, it would be extremely important to understand the mechanism. It will allow optimization of the laser parameter space and will aid the long-term safety of INS. Further work to understand the effect of beam quality (e.g. focused beam vs. collimated beam) on stimulation will aid in the design of efficient research and clinical stimulators. Standardized measurements of the spot size, using the knife-edge technique, and energy should also accompany any INS research.

Most of the work, to date, has been executed *in vivo* and, as such, it would be useful to determine any differences with INS *in vitro*. Many neural systems and neuron types have yet to be stimulated with infrared radiation and it is unknown if INS will work on all neurons or only a subset. While there are many questions yet to be answered, INS provides a unique application of biomedical optics that may foster significant advances in neuroscience.

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Abbreviations

δ	penetration depth
λ	wavelength
τ_p	pulse duration
f	repetition rate
E	irradiance
H	radiant exposure
P	power
Q	radiant energy
ABR	auditory brainstem response
AP	action potential
CAP	compound action potential
CmAP	compound muscle action potential
CnAP	compound nerve action potential
cw	continuous wave
FEL	free-electron laser
HeNe	helium-neon
Ho:YAG	holmium-doped: yttrium aluminum garnet
INS	infrared neural stimulation
Nd:YAG	neodymium-doped:YAG
ROC	receiver operating characteristics
TRPV	transient receptor potential vanilloid

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Figure 1.

(online color at: www.lpr-journal.org) (a) Wavelength dependence of stimulation. The free-electron laser (FEL) was used to irradiate four different nerves, three times each, at six wavelengths, 2.1, 3.0, 4.0, 4.5, 5.0, and 6.1 μm . The wavelengths were selected near a relative peak or valley of the water absorption (solid line). The numbers on the x -axis represent the corresponding wavelengths for the bar graph. The water absorption curve is overlaid to match up with the wavelengths for the bar graph. (b) Log-log plot of ablation and stimulation radiant exposure thresholds as a function of the water absorption coefficient for each wavelength. The figure has been published previously in the Journal of Biomedical Optics 10/6, 064003, 2005 and is reproduced with permission from SPIE Publications.

Figure 2.

Compound muscle action potentials (CmAP) recorded in the m. levator nasolabialis after stimulation of the facial nerve trunk with 70 μA current amplitude and 1.13 J/cm^2 radiant exposure. Note, optical radiation and electrical current generated similar response amplitudes. When examining the latency (delay time) between onset of the stimulus and onset of the evoked response, some differences arise between the various neural systems. For INS of the facial nerve, the optically evoked latency is longer than the latency for electrical stimulation. For the sciatic nerve (data not shown here), there was reportedly no difference in latency between an optically evoked compound nerve action potential (CnAP) and an electrically evoked CnAP. There is currently no explanation for the discrepancy in results. The figure has been published previously in *Laryngoscope* 117, 1641–1647, 2007 and is reproduced with permission from Lippincott Williams & Wilkins.

Figure 3.

Compound muscle action potentials (CmAPs) measured in three facial muscles. Left column: CmAP response evoked by an electrical stimulus ($70\ \mu\text{A}$) on the facial nerve trunk. Four right columns: CmAP responses to INS ($0.88\ \text{J}/\text{cm}^2$) at the same stimulation site for electrical stimulation. Both CmAPs are an average of 10 recordings. After the first recording (nerve center) the optical fiber was moved by $50\ \mu\text{m}$ steps, radially away from the nerve center. After each step, additional measurements were performed. Note, the isolated maximal response of the m. levator nasolabialis after moving the fiber $50\ \mu\text{m}$ from the initial nerve center. The figure has been published previously in *Laryngoscope* 117, 1641–1647, 2007 and is reproduced with permission from Lippincott Williams @ Wilkins.

Figure 4.

Input/output (I/O) curves recorded in response to laser stimulation of the gerbil cochlea. (a) The data show the amplitude of the evoked compound action potential (CAP) as the input radiant exposure is varied for a 5- μ s long optical pulse. There is a monotonic increase in evoked CAP amplitude with increasing radiant exposure. (b) The I/O function for a 30- μ s long pulse reveals a monotonic increase in CAP amplitude with increasing radiant exposure, followed by an apparent plateau in CAP amplitude above 15 mJ/cm². The lack of a plateau in CAP amplitude in (a) is likely due to a lower maximum radiant energy of the laser at the shorter pulse duration. In both panels, data from individual animals ($n = 11$) are represented by open squares and the mean is shown with the standard error. The figure has been published previously in the *Biophysical Journal* 117, 1641–1647, 2007 and is reproduced with permission from Cell Press Elsevier Inc.

Figure 5.

(online color at: www.lpr-journal.org) The left graphs provide examples for response areas recorded in the guinea pig inferior colliculus to cochlear INS. Color scheme is based on the calculation of the cumulative d-prime number, a measure for the activation. The x -axis is determined by the tonotopic map (acoustic) of the inferior colliculus penetrating electrode prior to conducting the optical stimulation experiments. Right panel: Using plots as shown in the upper panels, a quality factor, Q_{10dB} , was calculated as a measure of the selectivity of optical stimulation or acoustical stimulation. The tonotopically mapped location with the lowest optical or acoustical stimulation threshold (the tip) is determined, and the width of the response area (measured in Hz) is calculated 10 dB above the tip. Q_{10dB} is the ratio of the tonotopic frequency at the tip to the width of the response area. The symbols in the right panel show the average Q_{10dB} for stimulation with optical radiation (open circles) and for acoustical stimulation (filled circles). The Q_{10dB} is similar for both stimulation methods.

Figure 6.

Photonic stimulation of the superior semicircular canal. Radiation wavelength was 1842 nm, radiation energy = 2mJ irradiated through the overlying bone. The bottom trace, in which the laser radiation was blocked, demonstrates that the observed waveforms were not merely artifact. The laser pulse begins at $t = 0$. The figure has been published previously in Proc. SPIE 7180, 71800R-3, 2009) and is reproduced with permission from SPIE Publications.

Figure 7.

In the left panel the amplitude of the compound action potentials (CAPs) evoked from the guinea pig cochlea is plotted versus the radiant energy. Each trace in the graph represents a different laser pulse duration. Longer pulses appear to be less efficient. In the right panel the same data are plotted versus the power per pulse. The traces for the different pulse durations overlay. It appears that the transient of the optical pulse is more important than the total energy.