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Nanoparticle-Mediated Upconversion of Near-Infrared Light: A Step Closer to Optogenetic Neuromodulation in Humans

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Optogenetics, by genetically engineering neurons to express light-reactive ion channels, allows for temporally and spatially precise modulation of neuronal populations [1]. Described in 2005 by Boyden, Deisseroth and colleagues, and recipient of the Nature Method of the Year in 2010, Brain Prize 2013 and Breakthrough Prize in 2016, optogenetics is now widely used in fundamental neuroscience to modulate brain networks in living mice, rats and non-human primates [2–5]. In humans, deep brain stimulation (DBS) is currently performed using high-frequency electrical stimulation, which modulates all elements in the vicinity (glia, neuron perikaria, dendrites, axons) through poorly understood mechanisms and requires the long-term implantation of electrodes in deep brain structures [6]. Optogenetics is potentially superior to electrical stimulation by allowing cell type specificity, bidirectional control of neuronal activity (inhibition, excitation) and recording of neuronal activity during stimulation [7–9]. Optogenetics has been successfully used to treat Parkinson's disease, chronic pain, refractory epilepsy and retinitis pigmentosa in different animal models [2]. However, therapeutic deep brain optogenetic neuromodulation in humans is limited by a number of practical challenges, notably the long-term safety of viral vectors used for genetic engineering [8–11], the long-term expression of light-reactive channels in neurons [12], the effective delivery and diffusion of light through brain tissue [13, 14], tissue damage by light-related overheating [13, 14] and the still rudimentary understanding of network dynamics in the human brain [7, 8, 15].

In a recent ground-breaking article published in the *Science* February 2018 edition, Chen et al. [16] validated a method for near-infrared DBS via upconversion nanoparticle-mediated optogenetics, offering the potential for minimally invasive optogenetic neuromodulation [17]. Previous attempts at deep brain optogenetics relied on the implantation of optical fibers or light-emitting diodes (LEDs) [1, 2]. Given the human brain's high lipidic content, light hardly penetrates depths of more than 1–2 mm [13]. Light intensity can only be safely increased to a certain level, over which it causes neuronal damage through overheating [12, 13]. Near-infrared wavelengths (650–1,350 nm) show the highest depths of penetration

due to substantially less absorption from hemoglobin, myoglobin and lipid, but fail to consistently activate light-reactive channels [13, 16]. However, lanthanide-doped upconversion nanoparticles can convert low-energy near-infrared photons to high-energy visible emission [18]. In their study, Chen et al. [16] injected an adeno-associated virus (AAV) encoding channel rhodopsin-2 (a nonspecific cation channel) as well as NaYF₄:Yb/Tm nanocrystals coated with silica in the mouse ventral tegmental area. Transcranial continuous-wave near-infrared laser pulses were sufficient to reach nanocrystals, which upconverted the signal to blue emission (optimal wavelengths to activate channel rhodopsin-2). Activation of channel rhodopsin-2 cation channels triggered membrane depolarization sufficient to generate photocurrents and evoke spikes in transfected neurons of the ventral tegmental area. Likewise, authors were able to inhibit hippocampal activity in a mouse model of chemically induced seizure, using AAV encoding archaerhodopsin (a light-reactive proton pump) as well as green-emitting NaYF₄:Yb/Er nanoparticles coated with silica. The transcranial near-infrared signal was locally upconverted to green light by nanoparticles, which activated the archaerhodopsin light-reactive proton pump, hence hyperpolarizing (inhibiting) hippocampal neurons and hereby stopping seizures induced by the excitotoxin kainic acid. Although a small fraction of nanoparticles was taken up by neurons and microglia, the authors demonstrated absence of nanoparticle-related cytotoxicity and absence of significant heating caused by near-infrared light or nanoparticle signal upconversion.

These findings have far-reaching applications in the future of functional neurosurgery. First, this implies that superficial optogenetic neuromodulation could be achieved without inserting optical fibers, hence reducing hardware-related complications (infection, displacement) and allowing to modulate multiple spatially distant brain regions. In refractory temporal lobe epilepsy, one could inject AAV encoding the light-reactive hyperpolarizing channel (archaerhodopsin or halorhodopsin) and upconverting nanoparticles in the epileptic focus, then implant recording electrodes in the subdural space and finally implant a near-infrared laser source in the epidural space, as a seal to the cranium burr hole. This would allow for a minimally invasive closed-loop seizure control system. It remains unknown whether near-infrared light penetration in human brain tissue would be sufficient for noninvasive deep brain optogenetics. Upconverting nanoparticles would nevertheless allow to optogenetically modulate larger brain volumes in deep brain nuclei. Furthermore, injecting blue-emitting nanoparticles coupled with AAV encoding channel rhodopsin-2 in a given brain region and green-emitting nanoparticles coupled with AAV encoding archaerhodopsin in another region would allow for concomitant excitation and inhibition of distinct neuronal populations using the same near-infrared signal. This would allow a much more sophisticated, flexible and efficient modulation of neural networks in a variety of disorders.

Apart from the aforementioned light delivery issues, multiple challenges remain in the translational journey towards optogenetic

neuromodulation in humans. First, the long-term safety of viral vectors used for genetic engineering has yet to be fully demonstrated. Early trials of gene therapy in the 1990s relied on adenoviruses and retroviruses, which caused serious adverse effects due to high immunogenicity [11]. On the other hand, AAV has emerged as one of the safest and most commonly used vectors for the delivery of therapeutic genes in the brain [10]. It has been approved by the FDA for gene therapy from 1995 and for gene therapy in the brain from 2005 [19], and was consistently shown not to induce any significant immune or inflammatory response [10, 11, 19]. It has been safely used for the gene delivery in Parkinson's disease gene therapy trials [20, 21] and is now under trial for optogenetic neuromodulation of retinal neurons in patients who are blind from retinitis pigmentosa [22] (NCT03326336, NCT02556736). Demonstrated safety in these human trials would provide compelling arguments for the safety of deep brain optogenetic neuromodulation in humans. Second, the stability and long-term expression of light-reactive channels in neurons remains to be validated. Patients could potentially require repeated injections to maintain stable expression of light-reactive channels over time. Maintaining the expression of light-reactive channels in safe ranges is also a potential challenge, as high levels of expression of any protein may adversely affect cell health [23]. Third, the effects of chronic optogenetic neuromodulation remain to be elucidated. Whereas acute light stimulation triggers instantaneous excitation/inhibition and behavior modulation, chronic stimulation may eventually change the transfected neurons' response to stimulation or induce long-term potentiation or depression [24–26]. Long-term optogenetic neuromodulation of nonhuman primates will help gaining a better understanding of these potential issues [27]. Fourth, the potential of optogenetics to target specific neuronal populations (e.g., dopaminergic, serotonergic) still has to fully materialize. Currently, promoters have been developed to target dopaminergic neurons, glutamatergic neurons, inhibitory interneurons and Purkinje cells in nonhuman primates [27], yet we can expect further development in promoter-based targeting of specific neuronal types in the coming years. Finally, experimentation of optogenetic neuromodulation in humans will require a much better understanding of neural network dynamics in normal and diseased states [7, 8]. However, previous success of electrical DBS in various disorders and equally various brain targets provide, in addition to optogenetic experiments in nonhuman primates, a strong theoretical basis for future optogenetic neuromodulation trials in humans.

Altogether, although many challenges remain before the first trials of optogenetic neuromodulation in humans, recent discovery of minimally invasive optogenetics through nanoparticle-mediated upconversion of near-infrared light is a significant breakthrough. By resolving many important issues related to light penetration, this new technology brings us a step closer to optogenetics being integrated in the functional neurosurgeon toolbox to treat disorders of movement, behavior and cognition.

Disclosure Statement

There are no conflicts of interest.

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