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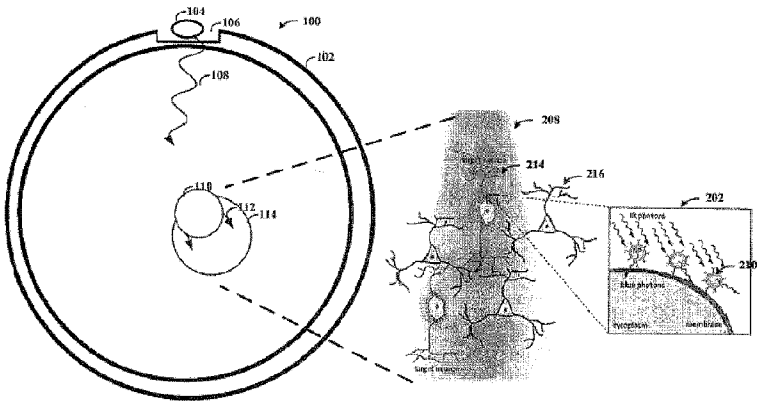

FIG. 1

FIG. 2

(57) Abstract: Provided herein are compositions comprising lanthanide-doped nanoparticles which upconvert electromagnetic radiation from infrared or near infrared wavelengths into the visible light spectrum. Also provided herein are methods activating light-responsive opsin proteins expressed on plasma membranes of neurons and selectively altering the membrane polarization state of the neurons using the light delivered by the lanthanide-doped nanoparticles.

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UPCONVERSION OF LIGHT FOR USE IN OPTOGENETIC METHODS

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 61/410,729 filed November 5, 2010, the disclosure of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

This application pertains to compositions comprising lanthanide-doped nanoparticles which upconvert electromagnetic radiation from infrared or near infrared wavelengths into the visible light spectrum and methods of using lanthanide-doped nanoparticles to deliver light to activate light-responsive opsin proteins expressed in neurons and selectively alter the membrane polarization state of the neurons.

BACKGROUND

Optogenetics is the combination of genetic and optical methods used to control specific events in targeted cells of living tissue, even within freely moving mammals and other animals, with the temporal precision (millisecond-timescale) needed to keep pace with functioning intact biological systems. The hallmark of optogenetics is the introduction of fast light-responsive opsin channel or pump proteins to the plasma membranes of target neuronal cells that allow temporally precise manipulation of neuronal membrane potential while maintaining cell-type resolution through the use of specific targeting mechanisms. Among the microbial opsins which can be used to investigate the function of neural systems are the halorhodopsins (NpHRs), used to promote membrane hyperpolarization when illuminated, and the channelrhodopsins, used to depolarize membranes upon exposure to light. In just a few short years, the field of optogenetics has furthered the fundamental scientific understanding of how specific cell types contribute to the function of biological tissues, such as neural circuits, *in vivo*. Moreover, on the clinical side, optogenetics-driven research has led to insights into the neurological mechanisms underlying complex mammalian behaviors such as anxiety, memory, fear, and addiction.

In spite of these advances, use of optogenetic methods in animals suffers from the significant drawback of requiring the animal to either be tethered to a light source or to have a light source surgically implanted into the animal. Moreover, when optogenetic methods are used to alter the function of neurons in the brain, a light source must be placed

in proximity to those neurons. This requires drilling a hole in the animal's skull and also presents practical difficulties when the brain region of interest is located deep within the brain itself. Since light poorly passes through neural tissue, this necessitates inserting a fiber optic light source into the brain, which can result in unintended damage to surrounding brain tissue.

What is needed, therefore, is a method to non-invasively deliver light to neurons located within the brain and the peripheral nervous system of animals expressing light-responsive opsin proteins on the plasma membranes of neural cells.

Throughout this specification, references are made to publications (e.g., scientific articles), patent applications, patents, etc., all of which are herein incorporated by reference in their entirety.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each claim of this application.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

SUMMARY OF THE INVENTION

Provided herein are compositions and methods for non-invasively delivering light to neurons expressing light-responsive opsin proteins on neural plasma membranes via the use of nanoparticles capable of upshifting electromagnetic radiation from wavelengths associated with the infrared (IR) or near infrared (NIR) spectrum into wavelengths associated with visible light.

In one aspect, the invention provides a method of depolarizing or hyperpolarizing the plasma membrane of a neural cell in an individual, wherein the method comprises: (a) placing a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and (b) exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (NIR) spectrum, wherein the electromagnetic radiation in the IR or NIR spectrum is upconverted into light in the visible spectrum by the nanoparticles, and wherein a light-responsive opsin is expressed on the plasma membrane of the neural cells and activation of the opsin by the light in the visible spectrum induces the depolarization or hyperpolarization

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of the plasma membrane; wherein the light-responsive opsin that induces depolarization comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:6, 7, 8, 9, 10 or 11; and wherein the light-responsive opsin that induces hyperpolarization comprises an amino acid sequence at least 85% identical to SEQ ID NO:4.

In another aspect, the invention provides a method of depolarizing or hyperpolarizing the plasma membrane of a neural cell in an individual, wherein the method comprises: (a) administering a polynucleotide encoding a light-responsive opsin to an individual, wherein the light-responsive protein is expressed on the plasma membrane of a neural cell in the individual and the opsin is capable of inducing membrane depolarization or hyperpolarization of the neural cell when illuminated with light; (b) administering a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and (c) exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (NIR) spectrum, wherein the electromagnetic radiation in the IR or NIR spectrum is upconverted into light in the visible spectrum and the activation of the opsin by the light in the visible spectrum induces the depolarization or hyperpolarization of the plasma membrane, wherein the light-responsive opsin that induces depolarization comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:6, 7, 8, 9, 10 or 11; and wherein the light-responsive opsin that induces hyperpolarization comprises an amino acid sequence at least 85% identical to SEQ ID NO:4.

Accordingly, provided herein is a method to depolarize the plasma membrane of a neural cell in an individual comprising: (a) placing a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and (b) exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (NIR) spectrum, wherein the electromagnetic radiation in the IR or MR spectrum is upconverted into light in the visible spectrum by the nanoparticles, and wherein a light-responsive opsin is expressed on the plasma membrane of the neural cells and activation of the opsin by the light in the visible spectrum induces the depolarization of the plasma membrane.

In other aspects, provided herein is a method to depolarize the plasma membrane of a neural cell in an individual comprising: (a) administering a polynucleotide encoding a light-responsive opsin to an individual, wherein the light-responsive protein is expressed on the plasma membrane of a neural cell in the individual, and the opsin is capable of inducing membrane depolarization of the neural cell when illuminated with light; (b) administering a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and (c) exposing

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the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (MR) spectrum, wherein the electromagnetic radiation in the IR or MR spectrum

is upconverted into light in the visible spectrum and the activation of the opsin by the light in the visible spectrum induces the depolarization of the plasma membrane.

In some aspects, provided herein is a method to hyperpolarize the plasma membrane of a neural cell in an individual comprising: (a) placing a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and (b) exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (NIR) spectrum, wherein the electromagnetic radiation in the IR or NIR spectrum is upconverted into light in the visible spectrum by the nanoparticles, and wherein a light-responsive opsin is expressed on the plasma membrane and activation of the opsin by the light in the visible spectrum induces the hyperpolarization of the plasma membrane.

In yet other aspects, provided herein is a method to hyperpolarize the plasma membrane of a neural cell in an individual comprising: (a) administering a polynucleotide encoding a light-responsive opsin to an individual, wherein the light-responsive protein is expressed on the plasma membrane of a neural cell in the individual, and the opsin is capable of inducing membrane hyperpolarization of the neural cell when illuminated with light; (b) administering a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and (c) exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (NIR) spectrum, wherein the electromagnetic radiation in the IR or NIR spectrum is upconverted into light in the visible spectrum and the activation of the opsin by the light in the visible spectrum induces the hyperpolarization of the plasma membrane.

The present disclosure is directed to apparatuses and methods involving upconversion for deep delivery of light *in vivo*. Aspects of the present disclosure relate generally to delivery of light to tissue *in vivo* using upconversion of near infrared light to the visible light spectrum and methods relating to the applications discussed herein.

Certain aspects of the present disclosure are directed to a light source that is implanted within living tissue. Nanoparticles from the nanoparticle solution anchor to a target cell population that includes cells expressing light responsive channels/opsins. The nanoparticles are configured to respond to receipt of light of a first wavelength by emitting light of a second, different wavelength. For example, the nanoparticles can upconvert received light and thereby emit light of a higher frequency.

Embodiments of the present disclosure are directed towards injection of a site of interest with a virus, caring an opsin gene and a nanoparticle solution. The virus causes a target cell population at the site of interest to express the opsin gene. Various different

light sources are possible. The use of different wavelengths can be particularly useful for facilitating the use of different (external) light sources, *e.g.*, as certain wavelengths exhibit corresponding decreases in absorption by tissue of the brain or otherwise.

Consistent with a particular embodiment of the present disclosure, a light-emitting diode ("LED") is placed on a portion of a skull that has been thinned. The LED is placed under the skin near the thinned portion of the skull, and the location and/or orientation of the LED is chosen, at least in part, based on the location of the target cell population. For example, the LED can be placed to reduce the distance between the LED and the target cell population and oriented accordingly.

In certain more specific aspects of the present disclosure, light from the LED travels through surrounding tissue to the nanoparticles. When (near) infrared light hits the nanoparticles, the nanoparticles absorb the infrared (IR) photons and emit visible photons. The visible photons are then absorbed by the opsins expressed within the target cell population causing a response therein (*e.g.,* triggering neural excitation or inhibition).

The LED can be powered by a battery similar to those used for pacemakers. The LED can emit light in the infrared spectrum, and particularly between 700nm-1000nm, which can travel through the skull and intervening tissue. The light emitted from the nanoparticles has a spectra centered between 450-550nm. The wavelength of the light emitted is dependent on characteristics of the nanoparticle.

The above overview is not intended to describe each illustrated embodiment or every implementation of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

Various example embodiments may be more completely understood in consideration of the following description and the accompanying drawings, in which:

FIG. 1 shows a cross section of a skull, consistent with an embodiment of the present disclosure.

FIG. 2 shows light delivery to target neurons, consistent with an embodiment of the present disclosure.

FIG. 3 depicts a system that uses multiple light sources, consistent with an embodiment of the present disclosure.

While the present disclosure is amenable to various modifications and alternative forms, specifics thereof have been shown by way of example in the drawings and will be described in detail. It should be understood, however, that the intention is not to limit the

present disclosure to the particular embodiments described. On the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the scope of the present disclosure including aspects defined in the claims.

DETAILED DESCRIPTION

This invention provides, *inter alia,* compositions and methods for delivering light to neural cells expressing one or more light-responsive opsin proteins on the plasma membranes of those neural cells. The inventors have discovered that nanoparticles doped with a lanthanide metal (for example, Gadolinium) that converts infrared (IR) or near infrared (NIR) electromagnetic radiation into wavelengths corresponding to the visible light spectrum can be used to activate light-responsive opsin proteins on the plasma membrane of a neural cell and selectively alter the membrane polarization state of the cell. Unlike visible light, IR or NIR electromagnetic energy readily penetrates biological tissues. For example, NIR can penetrate biological tissues for distances of up to 4 centimeters (Heyward & Dale Wagner, "*Applied Body Composition Assessment*", 2nd edition (2004), p. 100). Certain equations useful for calculating light penetration in tissue as a function of wavelength are disclosed in U.S. Pat. No. 7,043,287, the contents of which are incorporated herein by reference. Similarly, U.S. Patent Application Publication No. 2007/0027411 discloses that near infrared Low Level Laser Treatment light penetrates the body to a depth of between 3-5 cm. Therefore, use of IR or NIR sources of electromagnetic radiation in optogenetic methods can alleviate the need to place a light source in direct proximity to neural cells. In particular, for optogenetic techniques in the brain, use of lanthanide-doped nanoparticles in combination with IR or NIR electromagnetic energy can permit activation of the opsin protein without the need to puncture the skull or insert a fiber optic light source into the brain. Similarly, in the peripheral nervous system, opsin-expressing nerves can be activated via IR or NIR sources placed under the skin or worn against the skin.

General Techniques

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, cell biology, biochemistry, nucleic acid chemistry, immunology, and physiology, which are well known to those skilled in the art. Such techniques are explained fully in the literature, such as, *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook et al., 1989) and *Molecular Cloning: A Laboratory Manual,* third edition (Sambrook and Russel, 2001), (jointly

referred to herein as "Sambrook"); *Current Protocols in Molecular Biology* (F.M. Ausubel et al., eds., 1987, including supplements through 2001); *PCR: The Polymerase Chain Reaction,* (Mullis et al., eds., 1994); Harlow and Lane (1988), *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York; Harlow and Lane (1999), *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (jointly referred to herein as "Harlow and Lane"), Beaucage et al. eds., *Current Protocols in Nucleic Acid Chemistry*, John Wiley & Sons, Inc., New York, 2000), *Handbook of Experimental Immunology*, 4th edition (D. M. Weir & C. C. Blackwell, eds., Blackwell Science Inc., 1987), and *Gene Transfer Vectors for Mammalian Cells* (J. M. Miller & M. P. Calos, eds., 1987). Other useful references include Harrison's *Principles of Internal Medicine* (McGraw Hill; J. Isseleacher et al., eds.) and *Lanthanide Luminescence: Photophysical, Analytical and Biological Aspects* (Springer- Verlag, Berlin, Heidelberg; Hanninen & Harma, eds., 2011).

Definitions

As used herein, "infrared" or "near infrared" or "infrared light" or "near infrared light" refers to electromagnetic radiation in the spectrum immediately above that of visible light, measured from the nominal edge of visible red light at 0.74 μm, and extending to 300 μm. These wavelengths correspond to a frequency range of approximately 1 to 400 THz. In particular, "near infrared" or "near infrared light" also refers to electromagnetic radiation measuring 0.75-1.4 μm in wavelength, defined by the water absorption.

"Visible light" is defined as electromagnetic radiation with wavelengths between 380 nm and 750 nm. In general, "electromagnetic radiation," including light, is generated by the acceleration and deceleration or changes in movement (vibration) of electrically charged particles, such as parts of molecules (or adjacent atoms) with high thermal energy, or electrons in atoms (or molecules).

The term "nanoparticles" as used herein, can also refer to nanocrystals, nanorods, nanoclusters, clusters, particles, dots, quantum dots, small particles, and nanostructured materials. The term "nanoparticle" encompasses all materials with small size (generally, though not necessarily) less than 100 nm associated with quantum size effects.

An "individual" is a mammal including a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, mice and rats. Individuals also include companion animals including, but not limited to, dogs and cats. In some aspects, an individual is a non-human animal, such as a mammal. In another aspect, an individual is a human.

As used herein, the singular form "a", "an", and "the" includes plural references unless indicated otherwise.

It is intended that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

Lanthanide-doped Nanoparticles

In materials science, doping is commonly used to incorporate specific species of ions or atoms into a host lattice core structure to produce hybrid materials with new and useful properties. When synthesizing nanoparticles, doping can influence not only the size and shape of the particles, but also other properties, such as the ability to convert near infrared (NIR) excitation into a visible emission of light.

The lanthanide metals, or lanthanoids (also known as the "Rare Earth" metals), are elements of atomic number 57 (Lanthanum) through 71 (Lutetium), and often include Yttrium (atomic number 39) and Scandium (atomic number 21) because of their chemical similarities. Lanthanide ions exhibit unique luminescent properties, including the ability to convert near infrared long-wavelength excitation radiation into shorter visible wavelengths through a process known as photon upconversion. Lanthanides usually exist as trivalent cations, in which case their electronic configuration is (Xe) 4f, with n varying from 1 (Ce^{3+}) to 14 (Lu^{3+}). The transitions within the f-manifold are responsible for many of the photo-physical properties of the lanthanide ions, such as long-lived luminescence and sharp absorption and emission lines. The f-electrons are shielded from external perturbations by filled 5s and 5p orbitals, thus giving rise to line-like spectra. Additionally, the f-f electronic transitions of lanthanides are LaPorte forbidden, leading to long excited state lifetimes, in the micro- to millisecond range.

In some embodiments, any known method can be used to synthesize lanthanide-doped nanoparticles. Such methods are well known in the art (*See, e.g.*, Xu & Li, 2007, *Clin Chem.*, 53(8):1503-10; Wang et al., 2010, *Nature*, 463(7284):1061-5; U.S. Patent Application Publication Nos.: 2003/0030067 and 2010/0261263; and U.S. Patent No.: 7,550,201, the disclosures of each of which are incorporated herein by reference in their

entireties). For example, in some embodiments, lanthanide-doped nanorods can be synthesized with a $NaYF_4$ dielectric core, wherein a DI water solution (1.5 ml) of 0.3 g NaOH is mixed with 5 ml of ethanol and 5 ml of oleic acid under stirring. To the resulting mixture is selectively added 2 ml of $RECl_3$ (0.2 M, RE= Y, Yb, Er, Gd, Sm, Nd or La) and 1 ml of NH_4F (2 M). The solution is then transferred into an autoclave and heated at 200 °C for 2 h. Nanorods are then obtained by centrifugation, washed with water and ethanol several times, and finally re-dispersed in cyclohexane. In another non-limiting example, nanoparticles can be synthesized using 2 ml of $RECl_3$ (0.2 M, RE = Y, Yb, Er, Gd, or Tm) in methanol added to a flask containing 3 ml oleic acid and 7 ml of 1-octadecene. This solution is then heated to 160 °C for 30 min and cooled down to room temperature. Thereafter, a 5 ml methanol solution of NH_4F (1.6 mmol) and NaOH (1 mmol) is added and the solution is stirred for 30 min. After methanol evaporation, the solution is next heated to 300 °C under argon for 1.5 h and cooled down to room temperature. The resulting nanoparticles are precipitated by the addition of ethanol, collected by centrifugation, washed with methanol and ethanol several times, and finally re-dispersed in cyclohexane.

In one embodiment, the materials for the lanthanide-doped nanoparticle core can include a wide variety of dielectric materials. In various embodiments, the dielectric core can include lanthanide-doped oxide materials. Lanthanides include lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu). Other suitable dielectric core materials include non-lanthanide elements such as yttrium (Y) and scandium (Sc). Hence, suitable dielectric core materials include, but are not limited to, Y_2O_3, Y_2O_2S, $NaYF_4$, NaYbF4, Na doped YbF_3, YAG, YAP, Nd_2O_3, LaF_3, $LaCl_3$, La_2O_3, TiO_2, $LuPO_4$, YVO_4, YbF_3, YF_3, or SiO_2. In one embodiment, the dielectric nanoparticle core is $NaYF_4$. These dielectric cores can be doped with one or more Er, Eu, Yb, Tm, Nd, Tb, Ce, Y, U, Pr, La, Gd and other rare-earth species or a combination thereof. In one embodiment, the dielectric core material is doped with Gd. In another embodiment, the lanthanide-doped nanoparticle comprises $NaYF_4$:Yb/X/Gd, wherein X is Er, Tm, or Er/Tm. In some embodiments, the lanthanide-doped nanoparticles comprise a $NaYF_4$:Yb/Er (18/2 mol%) dielectric core doped with any of about 0 mol%, about 5 mol%, about 10 mol%, about 15 mol%, about 20 mol%, about 25 mol%, about 30 mol%, about 35 mol%, about 40 mol%, about 45 mol%, about 50 mol%, about 55 mol%, about or 60 mol% Gd^{3+} ions, inclusive, including any mol% in between these values. In other embodiments, the lanthanide-doped

nanoparticles comprise a $NaYF_4$:Yb/Er (18/2 mol%) dielectric core doped with any of about 0 mol%, about 5 mol%, about 10 mol%, about 15 mol%, about 20 mol%, about 25 mol%, or about 30 mol% Yb^{3+} ions, inclusive, including any mol% in between these values. In yet other embodiments, the lanthanide-doped nanoparticles comprise a $NaYF_4$:Yb/Er (18/2 mol%) dielectric core doped with any of about 0 mol%, about 5 mol%, about 10 mol%, about 15 mol%, about 20 mol%, about 25 mol%, or about 30 mol% Er^{3+} ions, inclusive, including any mol% in between these values. In other embodiments, the lanthanide-doped nanoparticles comprise a $NaYF_4$:Yb/Er (18/2 mol%) dielectric core doped with any of about 0 mol%, about 5 mol%, about 10 mol%, about 15 mol%, about 20 mol%, about 25 mol%, or about 30 mol% Tm^{3+} ions, inclusive, including any mol% in between these values. In another embodiment, the lanthanide-doped nanoparticle is selected from the group consisting of $NaYF_4$:Yb/Er/Gd (18/2/5 mol%), $NaYF_4$:Yb/Tm/Er/Gd (20/0.2/0.1/5 mol%), $NaYF_4$:Yb/Tm/Er/Gd (20/0.2/0.05/5 mol%), and $NaYF_4$:Yb/Tm/Gd (20/0.2/5 mol%).

In some aspects, the lanthanide-doped nanoparticles disclosed herein are conjugated to one or more delivery molecules to target them to one or more molecules expressed on the surface of a neural cell of interest (such as a neural cell expressing one or more light-responsive opsin proteins on its plasma membrane). These can include, without limitation, antibodies or fragments thereof, small molecules, as well as lectins or any other carbohydrate motif. The delivery molecules ensure that the lanthanide-doped nanoparticles remain in close proximity to the opsin proteins to permit activation upon upconversion of IR or NIR electromagnetic radiation. Antibody conjugation to nanoparticles is well-known in the art (*See, e.g.*, U.S. Patent Application Publication No.: 2010/0209352 and 2008/0267876, the contents of each of which are incorporated by reference herein in their entireties).

In another aspect, lanthanide-doped nanoparticles can be embedded or trapped within a biocompatible material which is surgically placed proximal to (such as adjacent to or around) the neural cell of interest (such as a neural cell expressing one or more light-responsive opsin proteins on its plasma membrane). In some embodiments, the biocompatible material is transparent, so that visible light produced by the upconversion of IR or NIR electromagnetic radiation by the lanthanide-doped nanoparticles can reach the light-responsive opsin proteins expressed on the surface of the neural cell of interest. The biocompatible materials used to embed or trap the lanthanide-doped nanoparticles can include, but are not limited to, Ioplex materials and other hydrogels such as those based on

2-hydroxyethyl methacrylate or acrylamide, and poly ether polyurethane ureas (PEUU) including Biomer (Ethicon Corp.), Avcothane (Avco-Everrett Laboratories), polyethylene, polypropylene, polytetrafluoroethylene (Gore-Tex™), poly(vinylchloride), polydimethylsiloxane, an ethylene-acrylic acid copolymer, knitted or woven Dacron, polyester-polyurethane, polyurethane, polycarbonatepolyurethane (Corethane™), polyamide (Nylon) and polystyrene. In one embodiment, the biocompatible material can be polydimethylsiloxane (PDMS). Additional compounds that may be used for embedding and/or trapping the lanthanide-doped nanoparticles disclosed herein are described in Kirk-Othmer, *Encyclopedia of Chemical Technology*, 3rd Edition 1982 (Vol. 19, pp. 275-313, and Vol. 18, pp. 219-2220), van der Giessen et al., 1996, *Circulation,* 94:1690-1997 (1996), U.S. Patent Application Publication No.: 2011/0054305, and U.S. Patent No.: 6,491,965, the contents of each which are incorporated herein by reference in their entireties.

Light-responsive Opsin Proteins

Provided herein are optogenetic-based compositions for selectively hyperpolarizing or depolarizing neurons of the central or peripheral nervous system. Optogenetics refers to the combination of genetic and optical methods used to control specific events in targeted cells of living tissue, even within freely moving mammals and other animals, with the temporal precision (millisecond-timescale) needed to keep pace with functioning intact biological systems. Optogenetics requires the introduction of fast light-responsive channel or pump proteins to the plasma membranes of target neuronal cells that allow temporally precise manipulation of neuronal membrane potential while maintaining cell-type resolution through the use of specific targeting mechanisms.

Light-responsive opsins that may be used in the present invention include opsins that induce hyperpolarization in neurons by light and opsins that induce depolarization in neurons by light. Examples of opsins are shown in **Tables 1** and **2** below.

Table 1 shows identified opsins for inhibition of cellular activity across the visible spectrum:

Opsin Type	Biological Origin	Wavelength Sensitivity	Defined action
NpHR	*Natronomonas pharaonis*	589nm max	Inhibition (hyperpolarization)
BR	*Halobacterium helobium*	570nm max	Inhibition (hyperpolarization)
AR	*Acetabulaira acetabulum*	518nm max	Inhibition (hyperpolarization)
GtR3	*Guillardia theta*	472nm max	Inhibition (hyperpolarization)
Mac	*Leptosphaeria maculans*	470-500nm max	Inhibition (hyperpolarization)
NpHr3.0	*Natronomonas pharaonis*	680nm utility 589nm max	Inhibition (hyperpolarization)
NpHR3.1	*Natronomonas pharaonis*	680nm utility 589nm max	Inhibition (hyperpolarization)

Table 2 shows identified opsins for excitation and modulation across the visible spectrum:

Opsin Type	Biological Origin	Wavelength Sensitivity	Defined action
VChR1	*Volvox carteri*	589nm utility 535nm max	Excitation (depolarization)
DChR	*Dunaliella salina*	500nm max	Excitation (depolarization)
ChR2	*Chlamydomonas reinhardtii*	470nm max 380-405nm utility	Excitation (depolarization)
ChETA	*Chlamydomonas reinhardtii*	470nm max 380-405nm utility	Excitation (depolarization)
SFO	*Chlamydomonas reinhardtii*	470nm max 530nm max	Excitation (depolarization) Inactivation
SSFO	*Chlamydomonas reinhardtii*	445nm max 590nm; 390-400nm	Step-like activation (depolarization) Inactivation
C1V1	*Volvox carteri and Chlamydomonas reinhardtii*	542nm max	Excitation (depolarization)
C1V1 E122	*Volvox carteri and Chlamydomonas reinhardtii*	546nm max	Excitation (depolarization)
C1V1 E162	*Volvox carteri and Chlamydomonas reinhardtii*	542nm max	Excitation (depolarization)
C1V1 E122/E162	*Volvox carteri and Chlamydomonas reinhardtii*	546nm max	Excitation (depolarization)

As used herein, a light-responsive opsin (such as NpHR, BR, AR, GtR3, Mac, ChR2, VChR1, DChR, and ChETA) includes naturally occurring protein and functional variants, fragments, fusion proteins comprising the fragments, or the full length protein. For example, the signal peptide may be deleted. A variant may have an amino acid sequence at least about any of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the naturally occurring protein sequence. A functional variant may have the same or similar hyperpolarization function or depolarization function as the naturally occurring protein.

Enhanced intracellular transport amino acid motifs

The present disclosure provides for the modification of light-responsive opsin proteins expressed in a cell by the addition of one or more amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells. Light-responsive opsin proteins having components derived from evolutionarily simpler organisms may not be expressed or tolerated by mammalian cells or may exhibit impaired subcellular localization when expressed at high levels in mammalian cells. Consequently, in some embodiments, the light- responsive opsin proteins expressed in a cell can be fused to one or more amino acid sequence motifs selected from the group consisting of a signal peptide, an endoplasmic reticulum (ER) export signal, a membrane trafficking signal, and/or an N-terminal golgi export signal. The one or more amino acid sequence motifs which enhance light- responsive opsin protein transport to the plasma membranes of mammalian cells can be fused to the N-terminus, the C-terminus, or to both the N- and C-terminal ends of the light- responsive opsin protein. Optionally, the light- responsive opsin protein and the one or more amino acid sequence motifs may be separated by a linker. In some embodiments, the light- responsive opsin protein can be modified by the addition of a trafficking signal (ts) which enhances transport of the protein to the cell plasma membrane. In some embodiments, the trafficking signal can be derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In other embodiments, the trafficking signal can comprise the amino acid sequence KSRITSEGEYIPLDQIDINV.

Additional protein motifs which can enhance light- responsive opsin protein transport to the plasma membrane of a cell are described in U.S. Patent Application Publication No. 2009/0093403, which is incorporated herein by reference in its entirety. In some embodiments, the signal peptide sequence in the protein can be deleted or substituted with a signal peptide sequence from a different protein.

Light-responsive chloride pumps

In some aspects, the light-responsive opsin proteins described herein are light-responsive chloride pumps. In some aspects of the methods provided herein, one or more members of the Halorhodopsin family of light-responsive chloride pumps are expressed on the plasma membranes of neurons of the central and peripheral nervous systems.

In some aspects, said one or more light-responsive chloride pump proteins expressed on the plasma membranes of nerve cells of the central or peripheral nervous systems can be derived from *Natronomonas pharaonis*. In some embodiments, the light-responsive chloride pump proteins can be responsive to amber light as well as red light and can mediate a hyperpolarizing current in the interneuron when the light-responsive chloride pump proteins are illuminated with amber or red light. The wavelength of light which can activate the light-responsive chloride pumps can be between about 580 and about 630 nm. In some embodiments, the light can be at a wavelength of about 590 nm or the light can have a wavelength greater than about 630 nm (*e.g.* less than about 740 nm). In another embodiment, the light has a wavelength of around 630 nm. In some embodiments, the light-responsive chloride pump protein can hyperpolarize a neural membrane for at least about 90 minutes when exposed to a continuous pulse of light. In some embodiments, the light-responsive chloride pump protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1. Additionally, the light-responsive chloride pump protein can comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-responsive protein to regulate the polarization state of the plasma membrane of the cell. In some embodiments, the light-responsive chloride pump protein contains one or more conservative amino acid substitutions. In some embodiments, the light-responsive protein contains one or more non-conservative amino acid substitutions. The light-responsive protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to hyperpolarize the plasma membrane of a neuronal cell in response to light.

Additionally, in other aspects, the light-responsive chloride pump protein can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1 and an endoplasmic reticulum (ER) export signal. This ER export signal can be fused to the C-

terminus of the core amino acid sequence or can be fused to the N-terminus of the core amino acid sequence. In some embodiments, the ER export signal is linked to the core amino acid sequence by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments, the ER export signal can comprise the amino acid sequence FXYENE, where X can be any amino acid. In another embodiment, the ER export signal can comprise the amino acid sequence VXXSL, where X can be any amino acid. In some embodiments, the ER export signal can comprise the amino acid sequence FCYENEV.

In other aspects, the light-responsive chloride pump proteins provided herein can comprise a light-responsive protein expressed on the cell membrane, wherein the protein comprises a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1 and a trafficking signal (*e.g.*, which can enhance transport of the light-responsive chloride pump protein to the plasma membrane). The trafficking signal may be fused to the C-terminus of the core amino acid sequence or may be fused to the N-terminus of the core amino acid sequence. In some embodiments, the trafficking signal can be linked to the core amino acid sequence by a linker which can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments, the trafficking signal can be derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In other embodiments, the trafficking signal can comprise the amino acid sequence KSRITSEGEYIPLDQIDINV.

In some aspects, the light-responsive chloride pump protein can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1 and at least one (such as one, two, three, or more) amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells selected from the group consisting of an ER export signal, a signal peptide, and a membrane trafficking signal. In some embodiments, the light-responsive chloride pump protein comprises an N-terminal signal peptide, a C-terminal ER Export signal, and a C-terminal trafficking signal. In some embodiments, the

C-terminal ER Export signal and the C-terminal trafficking signal can be linked by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker can also further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments the ER Export signal can be more C-terminally located than the trafficking signal. In other embodiments the trafficking signal is more C-terminally located than the ER Export signal. In some embodiments, the signal peptide comprises the amino acid sequence MTETLPPVTESAVALQAE. In another embodiment, the light-responsive chloride pump protein comprises an amino acid sequence at least 95% identical to SEQ ID NO:2.

Moreover, in other aspects, the light-responsive chloride pump proteins can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1, wherein the N-terminal signal peptide of SEQ ID NO:1 is deleted or substituted. In some embodiments, other signal peptides (such as signal peptides from other opsins) can be used. The light-responsive protein can further comprise an ER transport signal and/or a membrane trafficking signal described herein. In some embodiments, the light-responsive chloride pump protein comprises an amino acid sequence at least 95% identical to SEQ ID NO:3.

In some embodiments, the light-responsive opsin protein is a NpHR opsin protein comprising an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the sequence shown in SEQ ID NO:1. In some embodiments, the NpHR opsin protein further comprises an endoplasmic reticulum (ER) export signal and/or a membrane trafficking signal. For example, the NpHR opsin protein comprises an amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1 and an endoplasmic reticulum (ER) export signal. In some embodiments, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1 is linked to the ER export signal through a linker. In some embodiments, the ER export signal comprises the amino acid sequence FXYENE, where X can be any amino acid. In another embodiment, the ER export signal comprises the amino acid sequence VXXSL, where X can be any amino acid. In some embodiments, the ER export signal comprises the amino acid sequence FCYENEV. In some embodiments, the NpHR opsin protein comprises an amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER

export signal, and a membrane trafficking signal. In other embodiments, the NpHR opsin protein comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, the ER export signal, and the membrane trafficking signal. In other embodiments, the NpHR opsin protein comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, the membrane trafficking signal, and the ER export signal. In some embodiments, the membrane trafficking signal is derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In some embodiments, the membrane trafficking signal comprises the amino acid sequence K S R I T S E G E Y I P L D Q I D I N V. In some embodiments, the membrane trafficking signal is linked to the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1 by a linker. In some embodiments, the membrane trafficking signal is linked to the ER export signal through a linker. The linker may comprise any of 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments, the light-responsive opsin protein further comprises an N-terminal signal peptide. In some embodiments, the light-responsive opsin protein comprises the amino acid sequence of SEQ ID NO:2. In some embodiments, the light-responsive opsin protein comprises the amino acid sequence of SEQ ID NO:3.

Also provided herein are polynucleotides encoding any of the light-responsive chloride ion pump proteins described herein, such as a light-responsive protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal. In another embodiment, the polynucleotides comprise a sequence which encodes an amino acid at least 95% identical to SEQ ID NO:2 and/or SEQ ID NO:3. The polynucleotides may be in an expression vector (such as, but not limited to, a viral vector described herein). The polynucleotides may be used for expression of the light-responsive chloride ion pump proteins in neurons of the central or peripheral nervous systems.

Further disclosure related to light-responsive chloride pump proteins can be found in U.S. Patent Application Publication Nos: 2009/0093403 and 2010/0145418 as well as in International Patent Application No: PCT/US2011/028893, the disclosures of each of which are hereby incorporated by reference in their entireties.

Light-responsive proton pumps

In some aspects, the light-responsive opsin proteins described herein are light-responsive proton pumps. In some aspects of the compositions and methods provided herein, one or more light-responsive proton pumps are expressed on the plasma membranes of neurons of the central or peripheral nervous systems.

In some embodiments, the light-responsive proton pump protein can be responsive to blue light and can be derived from *Guillardia theta*, wherein the proton pump protein can be capable of mediating a hyperpolarizing current in the cell when the cell is illuminated with blue light. The light can have a wavelength between about 450 and about 495 nm or can have a wavelength of about 490 nm. In another embodiment, the light-responsive proton pump protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4. The light-responsive proton pump protein can additionally comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-responsive proton pump protein to regulate the polarization state of the plasma membrane of the cell. Additionally, the light-responsive proton pump protein can contain one or more conservative amino acid substitutions and/or one or more non-conservative amino acid substitutions. The light-responsive proton pump protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to hyperpolarize the plasma membrane of a neuronal cell in response to light.

In other aspects of the methods disclosed herein, the light-responsive proton pump protein can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4 and at least one (such as one, two, three, or more) amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells selected from the group consisting of a signal peptide, an ER export signal, and a membrane trafficking signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide and a C-terminal ER export signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide and a C-terminal trafficking signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide, a C-terminal ER Export signal, and a C-terminal trafficking signal. In some embodiments, the light-responsive proton pump protein

comprises a C-terminal ER Export signal and a C-terminal trafficking signal. In some embodiments, the C-terminal ER Export signal and the C-terminal trafficking signal are linked by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments the ER Export signal is more C-terminally located than the trafficking signal. In some embodiments the trafficking signal is more C-terminally located than the ER Export signal.

Also provided herein are isolated polynucleotides encoding any of the light-responsive proton pump proteins described herein, such as a light-responsive proton pump protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4. Also provided herein are expression vectors (such as a viral vector described herein) comprising a polynucleotide encoding the proteins described herein, such as a light-responsive proton pump protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4. The polynucleotides may be used for expression of the light-responsive proton pumps in neural cells of the central or peripheral nervous systems.

Further disclosure related to light-responsive proton pump proteins can be found in International Patent Application No. PCT/US2011/028893, the disclosure of which is hereby incorporated by reference in its entirety.

Light-activated cation channel proteins

In some aspects, the light-responsive opsin proteins described herein are light-activated cation channel proteins. In some aspects of the methods provided herein, one or more light-activated cation channels can be expressed on the plasma membranes of the neural cells of the central or peripheral nervous systems.

In some aspects, the light-activated cation channel protein can be derived from *Chlamydomonas reinhardtii*, wherein the cation channel protein can be capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In another embodiment, the light-activated cation channel protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:5. The light used to activate the light-activated cation channel protein derived from *Chlamydomonas reinhardtii* can have a

wavelength between about 460 and about 495 nm or can have a wavelength of about 480 nm. Additionally, the light can have an intensity of at least about 100 Hz. In some embodiments, activation of the light-activated cation channel derived from *Chlamydomonas reinhardtii* with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the light-activated cation channel. The light-activated cation channel protein can additionally comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-activated cation channel protein to regulate the polarization state of the plasma membrane of the cell.

Additionally, the light-activated cation channel protein can contain one or more conservative amino acid substitutions and/or one or more non-conservative amino acid substitutions. The light-activated proton pump protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to depolarize the plasma membrane of a neuronal cell in response to light.

Further disclosure related to light-activated cation channel proteins can be found in U.S. Patent Application Publication No. 2007/0054319 and International Patent Application Publication Nos. WO 2009/131837 and WO 2007/024391, the disclosures of each of which are herby incorporated by reference in their entireties.

Step function opsins and stabilized step function opsins

In other embodiments, the light-activated cation channel protein can be a step function opsin (SFO) protein or a stabilized step function opsin (SSFO) protein that can have specific amino acid substitutions at key positions throughout the retinal binding pocket of the protein. In some embodiments, the SFO protein can have a mutation at amino acid residue C128 of SEQ ID NO:5. In other embodiments, the SFO protein has a C128A mutation in SEQ ID NO:5. In other embodiments, the SFO protein has a C128S mutation in SEQ ID NO:5. In another embodiment, the SFO protein has a C128T mutation in SEQ ID NO:5. In some embodiments, the SFO protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:6.

In some embodiments, the SSFO protein can have a mutation at amino acid residue D156 of SEQ ID NO:5. In other embodiments, the SSFO protein can have a mutation at both amino acid residues C128 and D156 of SEQ ID NO:5. In one embodiment, the SSFO protein has an C128S and a D156A mutation in SEQ ID NO:5. In another embodiment,

the SSFO protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:7.

In some embodiments the SFO or SSFO proteins provided herein can be capable of mediating a depolarizing current in the cell when the cell is illuminated with blue light. In other embodiments, the light can have a wavelength of about 445 nm. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the SFO or SSFO protein with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the SFO or SSFO protein. In some embodiments, each of the disclosed step function opsin and stabilized step function opsin proteins can have specific properties and characteristics for use in depolarizing the membrane of a neuronal cell in response to light.

Further disclosure related to SFO or SSFO proteins can be found in International Patent Application Publication No. WO 2010/056970 and U.S. Provisional Patent Application Nos. 61/410,704 and 61/511,905, the disclosures of each of which are herby incorporated by reference in their entireties.

C1V1 chimeric cation channels

In other embodiments, the light-activated cation channel protein can be a C1V1 chimeric protein derived from the VChR1 protein of *Volvox carteri* and the ChR1 protein from *Chlamydomonas reinhardti*, wherein the protein comprises the amino acid sequence of VChR1 having at least the first and second transmembrane helices replaced by the first and second transmembrane helices of ChR1; is responsive to light; and is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments, the C1V1 protein can further comprise a replacement within the intracellular loop domain located between the second and third transmembrane helices of the chimeric light responsive protein, wherein at least a portion of the intracellular loop domain is replaced by the corresponding portion from ChR1. In another embodiment, the portion of the intracellular loop domain of the C1V1 chimeric protein can be replaced with the corresponding portion from ChR1 extending to amino acid residue A145 of the ChR1. In other embodiments, the C1V1 chimeric protein can further comprise a replacement within the third transmembrane helix of the chimeric light responsive protein, wherein at least a portion of the third transmembrane helix is replaced by the corresponding sequence of ChR1. In yet another embodiment, the portion of the intracellular loop domain of the C1V1 chimeric protein can be replaced with the corresponding portion from ChR1

extending to amino acid residue W163 of the ChR1. In other embodiments, the C1V1 chimeric protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:8.

In some embodiments, the C1V1 protein can mediate a depolarizing current in the cell when the cell is illuminated with green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 560 nm. In some embodiments, the light can have a wavelength of about 542 nm. In some embodiments, the C1V1 chimeric protein is not capable of mediating a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein is not capable of mediating a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the C1V1 chimeric protein with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1 chimeric protein. In some embodiments, the disclosed C1V1 chimeric protein can have specific properties and characteristics for use in depolarizing the membrane of a neuronal cell in response to light.

C1V1 chimeric mutant variants

In some aspects, the invention can include polypeptides comprising substituted or mutated amino acid sequences, wherein the mutant polypeptide retains the characteristic light-responsive nature of the precursor C1V1 chimeric polypeptide but may also possess altered properties in some specific aspects. For example, the mutant light-activated C1V1 chimeric proteins described herein can exhibit an increased level of expression both within an animal cell or on the animal cell plasma membrane; an altered responsiveness when exposed to different wavelengths of light, particularly red light; and/or a combination of traits whereby the chimeric C1V1 polypeptide possess the properties of low desensitization, fast deactivation, low violet-light activation for minimal cross-activation with other light-activated cation channels, and/or strong expression in animal cells.

Accordingly, provided herein are C1V1 chimeric light-activated proteins that can have specific amino acid substitutions at key positions throughout the retinal binding pocket of the VChR1 portion of the chimeric polypeptide. In some embodiments, the C1V1 protein can have a mutation at amino acid residue E122 of SEQ ID NO:7. In some embodiments, the C1V1 protein can have a mutation at amino acid residue E162 of SEQ ID NO:7. In other embodiments, the C1V1 protein can have a mutation at both amino acid

residues E162 and E122 of SEQ ID NO:7. In other embodiments, the C1V1 protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11. In some embodiments, each of the disclosed mutant C1V1 chimeric proteins can have specific properties and characteristics for use in depolarizing the membrane of an animal cell in response to light.

In some aspects, the C1V1-E122 mutant chimeric protein is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments the light can be green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 560 nm. In some embodiments, the light can have a wavelength of about 546 nm. In other embodiments, the C1V1-E122 mutant chimeric protein can mediate a depolarizing current in the cell when the cell is illuminated with red light. In some embodiments, the red light can have a wavelength of about 630 nm. In some embodiments, the C1V1-E122 mutant chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the C1V1-E122 mutant chimeric protein with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1-E122 mutant chimeric protein. In some embodiments, the disclosed C1V1-E122 mutant chimeric protein can have specific properties and characteristics for use in depolarizing the membrane of a neuronal cell in response to light.

In other aspects, the C1V1-E162 mutant chimeric protein is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments the light can be green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 535 nm. In some embodiments, the light can have a wavelength of about 542 nm. In other embodiments, the light can have a wavelength of about 530 nm. In some embodiments, the C1V1-E162 mutant chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the C1V1-E162 mutant chimeric protein with light having an intensity of 100

Hz can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1-E162 mutant chimeric protein. In some embodiments, the disclosed C1V1-E162 mutant chimeric protein can have specific properties and characteristics for use in depolarizing the membrane of a neuronal cell in response to light.

In yet other aspects, the C1V1-E122/E162 mutant chimeric protein is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments the light can be green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 560 nm. In some embodiments, the light can have a wavelength of about 546 nm. In some embodiments, the C1V1-E122/E162 mutant chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. In some embodiments, the C1V1-E122/E162 mutant chimeric protein can exhibit less activation when exposed to violet light relative to C1V1 chimeric proteins lacking mutations at E122/E162 or relative to other light-activated cation channel proteins. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the C1V1- E122/E162 mutant chimeric protein with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1- E122/E162 mutant chimeric protein. In some embodiments, the disclosed C1V1- E122/E162 mutant chimeric protein can have specific properties and characteristics for use in depolarizing the membrane of a neuronal cell in response to light.

Further disclosure related to C1V1 chimeric cation channels as well as mutant variants of the same can be found in U.S. Provisional Patent Application Nos. 61/410,736, 61/410,744, and 61/511,912, the disclosures of each of which are herby incorporated by reference in their entireties.

Polynucleotides

The disclosure also provides polynucleotides comprising a nucleotide sequence encoding a light-responsive opsin protein described herein. In some embodiments, the polynucleotide comprises an expression cassette. In some embodiments, the polynucleotide is a vector comprising the above-described nucleic acid(s). In some embodiments, the nucleic acid encoding a light-activated protein of the disclosure is operably linked to a promoter. Promoters are well known in the art. Any promoter that functions in the host cell can be used for expression of the light-responsive opsin proteins and/or any variant thereof of the present disclosure. In one embodiment, the promoter used

to drive expression of the light-responsive opsin proteins is a promoter that is specific to motor neurons. In another embodiment, the promoter used to drive expression of the light-responsive opsin proteins is a promoter that is specific to central nervous system neurons. In other embodiments, the promoter is capable of driving expression of the light-responsive opsin proteins in neurons of both the sympathetic and/or the parasympathetic nervous systems. Initiation control regions or promoters, which are useful to drive expression of the light-responsive opsin proteins or variant thereof in a specific animal cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these nucleic acids can be used. Examples of motor neuron-specific genes can be found, for example, in Kudo, et al., *Human Mol. Genetics*, 2010, 19(16): 3233-3253, the contents of which are hereby incorporated by reference in their entirety. In some embodiments, the promoter used to drive expression of the light-activated protein can be the Thy1 promoter, which is capable of driving robust expression of transgenes in neurons of both the central and peripheral nervous systems (*See, e.g.*, Llewellyn, et al., 2010, *Nat. Med.*, 16(10):1161-1166). In other embodiments, the promoter used to drive expression of the light-responsive opsin protein can be the EF1α promoter, a cytomegalovirus (CMV) promoter, the CAG promoter, the sinapsin promoter, or any other ubiquitous promoter capable of driving expression of the light-responsive opsin proteins in the peripheral and/or central nervous system neurons of mammals.

Also provided herein are vectors comprising a nucleotide sequence encoding a light-responsive opsin protein or any variant thereof described herein. The vectors that can be administered according to the present invention also include vectors comprising a nucleotide sequence which encodes an RNA (*e.g.*, an mRNA) that when transcribed from the polynucleotides of the vector will result in the accumulation of light-responsive opsin proteins on the plasma membranes of target animal cells. Vectors which may be used, include, without limitation, lentiviral, HSV, adenoviral, and andeno-associated viral (AAV) vectors. Lentiviruses include, but are not limited to HIV-1, HIV-2, SIV, FIV and EIAV. Lentiviruses may be pseudotyped with the envelope proteins of other viruses, including, but not limited to VSV, rabies, Mo-MLV, baculovirus and Ebola. Such vectors may be prepared using standard methods in the art.

In some embodiments, the vector is a recombinant AAV vector. AAV vectors are DNA viruses of relatively small size that can integrate, in a stable and site-specific manner, into the genome of the cells that they infect. They are able to infect a wide spectrum of cells without inducing any effects on cellular growth, morphology or differentiation, and

they do not appear to be involved in human pathologies. The AAV genome has been cloned, sequenced and characterized. It encompasses approximately 4700 bases and contains an inverted terminal repeat (ITR) region of approximately 145 bases at each end, which serves as an origin of replication for the virus. The remainder of the genome is divided into two essential regions that carry the encapsidation functions: the left-hand part of the genome, that contains the *rep* gene involved in viral replication and expression of the viral genes; and the right-hand part of the genome, that contains the *cap* gene encoding the capsid proteins of the virus.

AAV vectors may be prepared using standard methods in the art. Adeno-associated viruses of any serotype are suitable (*See, e.g.*, Blacklow, pp. 165-174 of "*Parvoviruses and Human Disease*" J. R. Pattison, ed. (1988); Rose, *Comprehensive Virology* 3:1, 1974; P. Tattersall "The Evolution of Parvovirus Taxonomy" in *Parvoviruses* (JR Kerr, SF Cotmore. ME Bloom, RM Linden, CR Parrish, Eds.) p5-14, Hudder Arnold, London, UK (2006); and DE Bowles, JE Rabinowitz, RJ Samulski "*The Genus Dependovirus*" (JR Kerr, SF Cotmore. ME Bloom, RM Linden, CR Parrish, Eds.) p15-23, Hudder Arnold, London, UK (2006), the disclosures of each of which are hereby incorporated by reference herein in their entireties). Methods for purifying for vectors may be found in, for example, U.S. Pat. Nos. 6,566,118, 6,989,264, and 6,995,006 and International Patent Application Publication No.: WO/1999/011764 titled "Methods for Generating High Titer Helper-free Preparation of Recombinant AAV Vectors", the disclosures of which are herein incorporated by reference in their entirety. Preparation of hybrid vectors is described in, for example, PCT Application No. PCT/US2005/027091, the disclosure of which is herein incorporated by reference in its entirety. The use of vectors derived from the AAVs for transferring genes *in vitro* and *in vivo* has been described (*See e.g.*, International Patent Application Publication Nos.: WO 91/18088 and WO 93/09239; U.S. Patent Nos.: 4,797,368, 6,596,535, and 5,139,941; and European Patent No.: 0488528, all of which are hereby incorporated by reference herein in their entireties). These publications describe various AAV-derived constructs in which the *rep* and/or *cap* genes are deleted and replaced by a gene of interest, and the use of these constructs for transferring the gene of interest *in vitro* (into cultured cells) or *in vivo* (directly into an organism). The replication defective recombinant AAVs according to the invention can be prepared by co-transfecting a plasmid containing the nucleic acid sequence of interest flanked by two AAV inverted terminal repeat (ITR) regions, and a plasmid carrying the AAV encapsidation genes (*rep* and *cap* genes), into a cell line that is infected with a human helper virus (for example, an

adenovirus). The AAV recombinants that are produced are then purified by standard techniques.

In some embodiments, the vector(s) for use in the methods of the invention are encapsidated into a virus particle (e.g. AAV virus particle including, but not limited to, AAV1, AAV2, AAV3, AAV4, AAV5,AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, and AAV16). Accordingly, the invention includes a recombinant virus particle (recombinant because it contains a recombinant polynucleotide) comprising any of the vectors described herein. Methods of producing such particles are known in the art and are described in U.S. Patent No. 6,596,535, the disclosure of which is hereby incorporated by reference in its entirety.

Delivery of Light-responsive Opsin Proteins and Lanthanide-doped Nanoparticles

In some aspects, polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV1 vector) can be delivered directly to neurons of the central or peripheral nervous system with a needle, catheter, or related device, using neurosurgical techniques known in the art, such as by stereotactic injection (*See, e.g.*, Stein et al., *J. Virol.*, 1999, 73:34243429; Davidson et al., *Proc. Nat. Acad. Sci. U.S.A.*, 2000, 97:3428-3432; Davidson et al., *Nat. Genet.*, 1993, 3:219-223; and Alisky & Davidson, *Hum. Gene Ther.*, 2000, 11:2315-2329, the contents of each of which are hereby incorporated by reference herein in their entireties) or fluoroscopy. In some embodiments, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV1 vector) can be delivered to neurons of the peripheral nervous system by injection into any one of the spinal nerves (such as the cervical spinal nerves, the thoracic spinal nerves, the lumbar spinal nerves, the sacral spinal nerves, and/or the coccygeal spinal nerves).

Other methods to deliver the light-responsive opsin proteins to the nerves of interest can also be used, such as, but not limited to, transfection with ionic lipids or polymers, electroporation, optical transfection, impalefection, or via gene gun.

In another aspect, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV2 vector) can be delivered directly to muscles innervated by the neurons of the peripheral nervous system. Because of the limitations inherent in injecting viral vectors directly into the specific cell bodies which innvervate particular muscles, researchers have attempted to deliver transgenes to peripheral neurons by injecting viral vectors directly into muscle. These experiments have shown that some viral serotypes such as adenovirus, AAV2, and Rabies glycoprotein-pseudotyped lentivirus

can be taken up by muscle cells and retrogradely transported to motor neurons across the neuromuscular synapse (*See, e.g.*, Azzouz et al., 2009, *Antioxid Redox Signal.*, 11(7):1523-34; Kaspar et al., 2003, *Science*, 301(5634):839-842; Manabe et al., 2002, *Apoptosis*, 7(4):329-334, the disclosures of each of which are herein incorporated by reference in their entireties).

Accordingly, in some embodiments, the vectors expressing the light-responsive opsin proteins disclosed herein (for example, an AAV2 vector) can be delivered to the neurons responsible for the innervation of muscles by direct injection into the muscle of interest.

The lanthanide-doped nanoparticles disclosed herein can be delivered to neurons expressing one or more light-responsive opsin proteins by any route, such as intravascularly, intracranially, intracerebrally, intramuscularly, intradermally, intravenously, intraocularly, orally, nasally, topically, or by open surgical procedure, depending upon the anatomical site or sites to which the nanoparticles are to be delivered. The nanoparticles can additionally be delivered by the same route used for delivery of the polynucleotide vectors expressing the light-responsive opsin proteins, such as any of those described above. The nanoparticles can also be administered in an open manner, as in the heart during open heart surgery, or in the brain during stereotactic surgery, or by intravascular interventional methods using catheters going to the blood supply of specific organs, or by other interventional methods.

Pharmaceutical compositions used for the delivery and/or storage of polynucleotides encoding the light-responsive opsin proteins disclosed herein and/or the lanthanide-doped nanoparticles disclosed herein can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in a number of sources which are well known and readily available to those skilled in the art. For example, Remington's *Pharmaceutical Sciences* (Martin E W, 1995, Easton Pa., Mack Publishing Company, 19th ed.) describes formulations which can be used in connection with the subject invention. Formulations suitable for parenteral administration include, for example, aqueous sterile injection solutions, which may contain antioxidants, buffers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be

stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use.

The lanthanide-doped nanoparticles may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the nanoparticles and/or cells can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion of the lanthanide-doped nanoparticles described herein can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like.

Sources of Infrared or Near Infrared Electromagnetic Radiation

Any device that is capable of producing a source of electromagnetic radiation having a wavelength in the infrared (IR) or near infrared (NIR) spectrum may be used to activate one or more light-responsive proteins expressed on the surface of a neuron in combination with the lanthanide-doped nanoparticles described herein. The IR or NIR source can be configured to provide optical stimulus to a specific target region of the brain. The IR or NIR source can additionally provide continuous IR or NIR electromagnetic radiation and/or pulsed IR or NIR electromagnetic radiation, and may be programmable to provide IR or NIR electromagnetic radiation in pre-determined pulse sequences.

In other aspects, the implantable IR or NIR source does not require physical tethering to an external power source. In some embodiments, the power source can be an internal battery for powering the IR or NIR source. In another embodiment, the implantable IR or NIR source can comprise an external antenna for receiving wirelessly transmitted electromagnetic energy from an external power source for powering the IR or NIR source. The wirelessly transmitted electromagnetic energy can be a radio wave, a microwave, or any other electromagnetic energy source that can be transmitted from an

external source to power the IR or NIR-generating source. In one embodiment, the IR or NIR source is controlled by an integrated circuit produced using semiconductor or other processes known in the art.

In some aspects, the implantable IR or NIR electromagnetic radiation source can be externally activated by an external controller. The external controller can comprise a power generator which can be mounted to a transmitting coil. In some embodiments of the external controller, a battery can be connected to the power generator, for providing power thereto. A switch can be connected to the power generator, allowing an individual to manually activate or deactivate the power generator. In some embodiments, upon activation of the switch, the power generator can provide power to the IR or NIR electromagnetic radiation source through electromagnetic coupling between the transmitting coil on the external controller and the external antenna of the implantable IR or NIR source. When radio-frequency magnetic inductance coupling is used, the operational frequency of the radio wave can be between about 1 and 20 MHz, inclusive, including any values in between these numbers (for example, about 1 MHz, about 2 MHz, about 3 MHz, about 4 MHz, about 5 MHz, about 6 MHz, about 7 MHz, about 8 MHz, about 9 MHz, about 10 MHz, about 11 MHz, about 12 MHz, about 13 MHz, about 14 MHz, about 15 MHz, about 16 MHz, about 17 MHz, about 18 MHz, about 19 MHz, or about 20 MHz). However, other coupling techniques may be used, such as an optical receiver or a biomedical telemetry system (*See, e.g.*, Kiourti, "Biomedical Telemetry: Communication between Implanted Devices and the External World, *Opticon1826*, (8): Spring, 2010).

In some aspects, the intensity of the IR or NIR electromagnetic radiation reaching the neural cells (such as neural cells expressing one or more light-responsive opsin proteins) produced by the IR or NIR electromagnetic radiation source has an intensity of any of about 0.05 mW/mm^2, 0.1 mW/mm^2, 0.2 mW/mm^2, 0.3 mW/mm^2, 0.4 mW/mm^2, 0.5 mW/mm^2, about 0.6 mW/mm^2, about 0.7 mW/mm^2, about 0.8 mW/mm^2, about 0.9 mW/mm^2, about 1.0 mW/mm^2, about 1.1 mW/mm^2, about 1.2 mW/mm^2, about 1.3 mW/mm^2, about 1.4 mW/mm^2, about 1.5 mW/mm^2, about 1.6 mW/mm^2, about 1.7 mW/mm^2, about 1.8 mW/mm^2, about 1.9 mW/mm^2, about 2.0 mW/mm^2, about 2.1 mW/mm^2, about 2.2 mW/mm^2, about 2.3 mW/mm^2, about 2.4 mW/mm^2, about 2.5 mW/mm^2, about 3 mW/mm^2, about 3.5 mW/mm^2, about 4 mW/mm^2, about 4.5 mW/mm^2, about 5 mW/mm^2, about 5.5 mW/mm^2, about 6 mW/mm^2, about 7 mW/mm^2, about 8

mW/mm^2, about 9 mW/mm^2, or about 10 mW/mm^2 , inclusive, including values in between these numbers.

In other aspects, the IR or NIR electromagnetic radiation produced by the IR or NIR electromagnetic radiation source can have a wavelength encompassing the entire infrared spectrum, such as from about 740 nm to about 300,000 nm. In other embodiments, the IR or NIR electromagnetic radiation produced by the IR or NIR electromagnetic radiation source can have a wavelength corresponding to the NIR spectrum, such as about 740 nm to about 1400 nm. In other embodiments, NIR electromagnetic radiation produced has a wavelength between 700 nm and 1000 nm.

In some aspects, an IR or NIR electromagnetic radiation source is used to hyperpolarize or depolarize the plasma membranes of neural cells (such as neural cells expressing one or more light-responsive opsin proteins) in the brain or central nervous system of an individual when used in combination with the lanthanide-doped nanoparticles disclosed herein. In some embodiments, the skull of the individual is surgically thinned in an area adjacent to the brain region of interest without puncturing the bone. The IR or NIR electromagnetic radiation source can then be placed directly over the thinned-skull region. In other embodiments, the IR or NIR electromagnetic radiation generator is implanted under the skin of the individual directly adjacent to the thinned skull region.

In some aspects, an IR or NIR electromagnetic radiation source is used to hyperpolarize or depolarize the plasma membranes of neural cells (such as neural cells expressing one or more light-responsive opsin proteins) in the peripheral nervous system of an individual when used in combination with the lanthanide-doped nanoparticles disclosed herein. In some embodiments, the IR or NIR electromagnetic radiation source is surgically implanted under the skin of the individual directly adjacent to the peripheral neural cell of interest. In other embodiments, the IR or NIR electromagnetic radiation source is placed against the skin directly adjacent to the peripheral neural cell of interest. In one embodiment, the IR or NIR electromagnetic radiation source is held against the skin in a bracelet or cuff configuration.

Examples of the IR or NIR electromagnetic radiation sources, particularly those small enough to be implanted under the skin, can be found in U.S. Patent Application Publication Nos.: 2009/0143842, 2011/0152969, 2011/0144749, and 2011/0054305, the disclosures of each of which are incorporated by reference herein in their entireties.

In still other aspects, the lanthanide-doped nanoparticles disclosed herein can be exposed to higher wavelength light in the visible spectrum (such as red light) to upconvert

the higher wavelength visible light into lower wavelength visible light (such as blue or green light). As described above, light passes through biological tissue poorly. However, when visible light does penetrate into tissues, it typically does so in higher wavelengths which correspond to red light (for example, between about 620 nm to 740 nm). Accordingly, the lanthanide-doped nanoparticles disclosed herein can additionally be used in combination with optical sources of visible light to upshift wavelengths corresponding to red light into wavelengths corresponding to green or blue light (for example, between about 440 nm and 570 nm). Examples of light stimulation devices, including light sources, can be found in International Patent Application Nos.: PCT/US08/50628 and PCT/US09/49936 and in Llewellyn et al., 2010, *Nat. Med.*, 16(10):161-165, the disclosures of each of which are hereby incorporated herein in their entireties.

Methods of the Invention

Depolarization of neural cells

Provided herein are methods to depolarize the plasma membrane of a neural cell in an individual comprising placing a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (NIR) spectrum, wherein the electromagnetic radiation in the IR or NIR spectrum is upconverted into light in the visible spectrum by the nanoparticles, and wherein a light-responsive opsin is expressed on the plasma membrane of the neural cells and activation of the opsin by the light in the visible spectrum induces the depolarization of the plasma membrane.

Also provided herein is a method to depolarize the plasma membrane of a neural cell in an individual comprising administering a polynucleotide encoding a light-responsive opsin to a neural cell in the brain of an individual, wherein the light-responsive protein is expressed on the plasma membrane of the neural cell and the opsin is capable of inducing membrane depolarization of the neural cell when illuminated with light administering a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near (IR) spectrum, wherein the electromagnetic radiation in the IR or near IR spectrum is upconverted into light in the visible spectrum and the activation of the opsin by the light in the visible spectrum induces the depolarization of the plasma membrane.

In some embodiments, the light-responsive opsin protein is ChR2, VChR1, or C1V1. In other embodiments, the light-responsive opsin protein is selected from the group consisting of SFO, SSFO, C1V1-E122, C1V1-E162, and C1V1-E122/E162.

The lanthanide metal can be ions or atoms from any of the lanthanide series of elements, such as Lanthanum, Cerium, Praseodymium, Neodymium, Promethium, Samarium, Europium, Gadolinium, Terbium, Dysprosium, Holmium, Erbium, Thulium, Ytterbium, or Lutetium. In other embodiments, the nanoparticles comprise NaYF4:Yb/X/Gd, wherein X is Er, Tm, or Er/Tm.

The electromagnetic radiation in the IR or near IR spectrum can be upconverted into light having a wavelength of about 450 nm to about 550 nm. The light can have wavelengths corresponding to red, yellow, amber, orange, green, or blue light. In some embodiments, the individual is a human or a non-human animal. In other embodiments, the neural cell is in the peripheral nervous system. In another embodiment, the neural cell is in the central nervous system.

Hyperpolarization of neural cells

Provided herein are methods to hyperpolarize the plasma membrane of a neural cell in an individual comprising placing a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (NIR) spectrum, wherein the electromagnetic radiation in the IR or NIR spectrum is upconverted into light in the visible spectrum by the nanoparticles, and wherein a light-responsive opsin is expressed on the plasma membrane of the neural cells and activation of the opsin by the light in the visible spectrum induces the hyperpolarization of the plasma membrane.

Also provided herein is a method to hyperpolarize the plasma membrane of a neural cell in an individual comprising administering a polynucleotide encoding a light-responsive opsin to a neural cell in the brain of an individual, wherein the light-responsive protein is expressed on the plasma membrane of the neural cell and the opsin is capable of inducing membrane depolarization of the neural cell when illuminated with light administering a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near (IR) spectrum, wherein the electromagnetic radiation in the IR or near IR spectrum is upconverted into light in the visible spectrum and the activation of the opsin by the light in the visible spectrum induces the hyperpolarization of the plasma membrane.

In some embodiments, the light-responsive opsin protein is an NpHR or a GtR3.

The lanthanide metal can be ions or atoms from any of the lanthanide series of elements, such as Lanthanum, Cerium, Praseodymium, Neodymium, Promethium, Samarium, Europium, Gadolinium, Terbium, Dysprosium, Holmium, Erbium, Thulium, Ytterbium, or Lutetium. In other embodiments, the nanoparticles comprise NaYF4:Yb/X/Gd, wherein X is Er, Tm, or Er/Tm.

The electromagnetic radiation in the IR or near IR spectrum can be upconverted into light having a wavelength of about 450 nm to about 550 nm. The light can have wavelengths corresponding to red, yellow, amber, orange, green, or blue light. In some embodiments, the individual is a human or a non-human animal. In other embodiments, the neural cell is in the peripheral nervous system. In another embodiment, the neural cell is in the central nervous system.

Kits

Also provided herein are kits comprising polynucleotides encoding a light-responsive opsin protein (such as any of the light-responsive opsin proteins described herein) and lanthanide-doped nanoparticles for use in any of the methods disclosed herein to alter the membrane polarization state of one or more neurons of the central and/or peripheral nervous system. In some embodiments, the kits further comprise an infrared or near infrared electromagnetic radiation source. In other embodiments, the kits further comprise instructions for using the polynucleotides and lanthanide-doped nanoparticles described herein. In still other embodiments, the lanthanide-doped nanoparticles described herein are embedded and/or trapped in a biocompatible material (such as any of the biocompatible materials described above).

EXEMPLARY EMBODIMENTS

Aspects of the present disclosure may be more completely understood in consideration of the detailed description of various embodiments of the present disclosure that follows in connection with the accompanying drawings. This description and the various embodiments are presented as follows:

The embodiments and specific applications discussed herein may be implemented in connection with one or more of the above-described aspects, embodiments and implementations, as well as with those shown in the figures and described below. Reference may also be made to Wang et al., 2010, *Nature*, 463(7284):1061-5, which is fully incorporated herein by reference. For further details on light responsive molecules and/or opsins, including methodology, devices and substances, reference may also be made

to the following background publications: U.S. Patent Publication No. 2010/0190229, entitled "System for Optical Stimulation of Target Cells" to Zhang et al.; U.S. Patent Publication No. 2007/0261127, entitled "System for Optical Stimulation of Target Cells" to Boyden et al. These applications form part of the provisional patent document and are fully-incorporated herein by reference. Consistent with these publications, numerous opsins can be used in mammalian cells *in vivo* and *in vitro* to provide optical stimulation and control of target cells. For example, when ChR2 is introduced into an electrically-excitable cell, such as a neuron, light activation of the ChR2 channelrhodopsin can result in excitation and/or firing of the cell. In instances when NpHR is introduced into an electrically-excitable cell, such as a neuron, light activation of the NpHR opsin can result in inhibition of firing of the cell. These and other aspects of the disclosures of the above-referenced patent applications may be useful in implementing various aspects of the present disclosure.

In various embodiments of the present disclosure, minimally invasive delivery of light, for example as can be useful for manipulation of neural circuits with optogenetics, using near infrared up-conversion nanocrystals, is achieved. This is used to avoid the implantation of light sources within living tissues, including, for example, a subject's brain. Mammalian tissue has a transparency window in near infrared part of the spectrum (700-1000nm). Accordingly, aspects of the present disclosure relate to the use of nanoparticles for the purpose of using (near) infrared light to deliver energy into the depth of a brain by converting the infrared light into visible wavelengths at a site of interest.

In certain embodiments, delivering visible wavelengths at a site of interest within the brain is achieved through a process of optical upconversion in Lanthanide-doped nanocrystals. During upconversion 3-4 photons are absorbed by the material which then emits one photon with the energy ~1.5-2 times the energy of absorbed photons. For example NaYF4:Yb/X/Gd nanocrystals can absorb 980 nm light and emit light with spectra centered between 450-550nm depending on the nature and relative content of dopants (X=Er, Tm, Er/Tm). For more information regarding modifying the light emitted from the nanoparticles, *see* Wang et al., *Nature*, 2010, 463(7284):1061-5, the disclosure of which is incorporated by reference herein in its entirety.

In certain embodiments a single step surgery is performed to modify a target cell population and provide nanoparticles to convert near infrared light to visible light that stimulates the modified target cell population. During the surgery, the surgeon injects both

an adeno-associated virus carrying an opsin gene and a nanoparticle solution to a site of interest.

The virus is optimized to only infect the target cell population. Similarly, the nanoparticles are functionalized with antibodies so that the nanoparticles anchor to the target cell population as well. In certain more specific embodiments the target cell population is a particular neuron type. After surgery is completed, a LED that emits near infrared light is placed on a thinned portion of the patient's skull, underneath the skin. A battery can also be implanted underneath the skin to power the LED. In certain embodiments the battery has characteristics similar to those of a pacemaker battery. A microcontroller can be used to control the battery to deliver energy to the LED at specified intervals, resulting in LED light pulses at specified intervals.

Certain aspects of the present disclosure are directed to the use of optogenetics *in vivo*. Optogenetics, applied *in vivo*, relies on light delivery to specific neuron populations that can be located deep within the brain. Mammalian tissue is highly absorptive and scatters light in the visible spectrum. However, near infrared light is able to penetrate to deep levels of the brain without excessive absorption or scattering.

Certain aspects of the present disclosure are directed to imbedding nanoparticles in the brain near target neurons. The nanoparticles can be lanthanide doped-nanoparticle. Nanoparticles doped with Lanthanides or with other dopants can be optimized with respect to a particular opsin's activation spectra. As discussed in more detail in Wang et al., *Nature*, 2010, 463(7284):1061-5, the disclosure of which is incorporated by reference herein in its entirety, the spectra of the light emitted from lanthanide-doped nanocrystals can be manipulated based on which dopants are used, and how much. Similarly, the light emitted from nanoparticles doped with other molecules can be manipulated based on the concentration of dopants.

The ability to provide different output spectra depending on the doping of nanoparticles allows for a non-invasive approach to acute neural manipulation. A light source, such as a LED can be mounted onto a thinned skull under the skin. Depending on the composition of nanoparticles, and the opsin delivered to the target neurons, aspects of the present disclosure can be used for neural excitation or silencing. Similarly, multiple neural populations may be controlled simultaneously through the use of various dopants and opsins in combination.

Turning to **FIG. 1**, a patient's head **100** is shown. A target (neural) cell population **114** includes light responsive molecules. These light responsive molecules

can include, but are not necessarily limited to, opsins derived from Channelrhodopsins *(e.g.* ChR1 or ChR2) or Halorhodopins (NpHR). The specific molecule can be tailored/selected based upon the desired effect on the target cell population and/or the wavelength at which the molecules respond to light.

Nanocrystals **110** are introduced near or at the target cell populate. Various embodiments of the present disclosure are directed toward methods and devices for positioning and maintaining positioning of the nanocrystals near the target cell population. Certain embodiments are directed toward anchoring the nanocrystals to cells of (or near) the target cell population using antibodies.

According to other example embodiments, a structure can be introduced that includes the nanocrystals. For instance, a mesh structure can be coated with the nanocrystals. The synthetic mesh can be constructed so as to allow the dendrites and axons to pass through the mess without allowing the entire neuron *(e.g.,* the cell body) to pass. One example of such a mesh has pores that are on the order of 3-7 microns in diameter and is made from polyethylene terephthalate. This mesh structure can be constructed with light-responsive cells/neurons contained therein and/or be placed near the target cell population, which includes the light- responsive cells. Consistent with another embodiment, one or more transparent capsules, each containing a solution of nanocrystals, can be positioned near the target cell populations.

Embodiments of the present disclosure are also directed toward various optical sources of stimulation. These sources can include, but are not limited to, external laser sources and light- emitting didoes (LEDs). Particular aspects of the present disclosure are directed toward the relatively low absorption and/or scattering/diffusion caused by intervening material when the light is at certain wavelengths *(e.g.,* (near) infrared). Accordingly, the light source can be externally located because of the ability to penetrate the tissue with little loss of optical intensity or power. Moreover, reduced diffusion can be particularly useful for providing a relatively-high spatial-precision for the delivery of the light. Thus, embodiments of the present disclosure are directed toward multiple target cell populations with respective nanocrystals that can be individually controlled using spatially-precise optical stimulus. For instance, the nanocrystals can be implanted in several locations within the brain. The light source can then be aimed at a respective and particular location. Multiple light sources can also be used for simultaneous stimulation of a plurality of locations.

Consistent with a particular embodiment of the present disclosure, the skull **102** has a thinned portion **106**. An LED **104** is located above the thinned portion of the skull and emits near infrared light **108**. When the IR hits nanocrystal **110**, it is absorbed. The nanocrystal emits visible light **112** in response to absorbing the IR light **108**. The visible light **112** is absorbed by modified cell **114**.

The system shown in **FIG. 1** allows for delivery of light to a target cell deep within a patient's brain tissue. The light responsive molecule can be specifically targeted to a neural cell type of interest. Similarly, the nanocrystals **112** are anchored to the neural cell with antibodies chosen based on the type of neural cell **114** being targeted.

Turning to **FIG. 2**, a group of neurons is illuminated with infrared light **208** between 700- 1000nm. Target neurons **214** express an opsin gene, allowing the neurons to be activated or inhibited, depending on which opsin, and what wavelength of light is absorbed by the neurons **214**. The target neurons **214** can be interspersed between other neurons **216**. As shown in inset **202**, target neurons **214** are coated with upconverting nanoparticles **210** that are anchored to the neural membrane via antibodies. The nanoparticles **210** absorb IR photons and emit visible photons that are then absorbed by opsins triggering neural activation.

The system of **FIG. 2** can be used with a variety of target neurons **214**. The opsin gene **215** expressed in the target neurons **214** is modified based on the target neuron. Similarly, the antibodies used to anchor the nanoparticles **210** to the target neuron membranes are modified to attach to a specific membrane type. As shown in inset **202**, the nanoparticles **210** are closely linked to the target neurons so that visible light photons emitted by the nanoparticles **210** are absorbed by the target neurons **214**.

FIG. 3 depicts a system that uses multiple light sources, consistent with an embodiment of the present disclosure. A patient has nanoparticles located at target locations **308-312**. The system includes light sources **302-306**, which can be configured to generate light at a frequency that is upconverted by the nanoparticles located at target locations **308-312**. Although three light sources are depicted, there can be any number of light sources. These light sources can be external to the patient (*e.g.*, a targeting system that directs several light sources using mechanical positioning), using embedded lights sources (*e.g.*, LEDs implanted on the skull) or combinations thereof. The target locations **308-312** include cells that have optically-responsive membrane molecules. These optically-responsive membrane molecules react to light at the upconverted frequency.

Nanoparticles located at the intersection **314** of the light from the different light sources **302-306** receive increased intensity of optical stimulus relative to other locations, including those locations within the path of light from a single light source. In this manner, the light intensity of each of the light sources can be set below a threshold level. When multiple light sources are directed at the same location, the threshold intensity level can be exceeded at the location. This allows for spatial control in three-dimensions and also allows for reduced inadvertent effects on non-targeted tissue. Consistent with one embodiment, the threshold level can be set according to an amount of light necessary to cause the desired effect (*e.g.,* excitation or inhibition) on the target cells. Consistent with other embodiments, the threshold level can be set to avoid adverse effects on non-targeted tissue (*e.g.,* heating).

The use of multiple light sources can also bring about a step-wise increase in light inntensity. For instance, a disease model could be tested by monitoring the effects of additional stimulation caused by the increase in light intensity. The use of independent light sources allows for relatively simple control over temporal and spatial increases or decreases. Consistent with other embodiments of the present disclosure, the spatial precision of the light sources can be varied between the different light sources. For example, a first light source can provide light that illuminates the entire target cell location. This allows for all cells within the population to be illuminated. A second light source can provide light having a focal point that illuminates less than all of the entire target cell location. The combination of the first and second (or more) light sources can be used to provide different levels of stimulation within the same cell population.

Embodiments of the present disclosure relate to the use of one or more light sources operating in a scanning mode. The light source(s) are aimed at specific locations within a target cell population. The effects of the stimulation can be monitored as the light source is used to scan or otherwise move within the target cell population. This can be particularly useful in connection with the three-dimensional control provided by the use of multiple light sources.

Various embodiments of the present disclosure are directed toward the use of nanocrystals that emit light at different wavelengths. This can be particularly useful when using multiple opsins having different light-absorption spectrums. The nanocrystals can be targeted toward different opsins and/or placed in the corresponding locations. While the present disclosure is amenable to various modifications and alternative forms, specifics thereof have been shown by way of example in the drawings and will be described in

further detail. It should be understood that the intention is not to limit the disclosure to the particular embodiments and/or applications described. On the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present disclosure.

EXAMPLES

Example 1: Use of lanthanide-doped nanoparticles in the use of optogenetics to hyperpolarize the cholinergic interneurons of the nucleus accumbens

The nucleus accumbens (NAc) is a collection of neurons that forms the main part of the ventral striatum. The NAc is thought to play an important role in the complex mammalian behaviors associated with reward, pleasure, laughter, addiction, aggression, fear, and the placebo effect. Cholinergic interneurons within the NAc constitute less than 1% of the local neural population, yet they project throughout the NAc and provide its only known cholinergic input. In this Example, an optogenetic approach using a light-responsive chloride pump protein in combination with lanthanide-doped nanoparticles is used to block action potential firing in these cells, with both high temporal resolution and high cell-type specificity. To express microbial opsin genes specifically in cholinergic interneurons, a transgenic mouse line expressing Cre recombinase is employed under the choline acetyltransferase (ChAT) promoter. A Cre-inducible adeno-associated virus (AAV) vector carrying a yellow-light gated third-generation chloride pump halorhodopsin (eNpHR3.0) gene fused in-frame with coding sequence for enhanced yellow fluorescent protein (eYFP) is stereotactically injected.

Specifically, mice are anesthetized and then placed in a stereotactic head apparatus. Surgeries are performed on 4-6 week old mice and ophthalmic ointment is applied throughout to prevent the eyes from drying. A midline scalp incision is made followed by a craniotomy, and then AAV vector is injected with a 10 µl syringe and a 34 gauge metal needle. The injection volume and flow rate (1µl at 0.15 µl/min) are controlled by an injection pump. Each NAc receives two injections (injection 1: AP 1.15mm, ML 0.8mm, DV -4.8mm; injection 2: AP 1.15mm, ML 0.8mm, DV -4.2mm). The virus injection and fiber position are chosen so that virtually the entire shell is stimulated.

Next, before withdrawing the needle, $NaYF_4$:Yb/Er/Gd, nanoparticles are injected into the Nac. Concentrations of 3.4, 8.5, or 17 nmoles of NaYF4:Yb/Er/Gd, nanoparticles are used. After injection of both the AAV vector and the lanthanide-doped nanoparticles is complete, the needle is left in place for 5 additional minutes and then very slowly withdrawn.

Following a recovery period, the mice are again anesthetized, the skulls of the mice are thinned and an NIR source of electromagnetic radiation is placed adjacent to the thinned skull-region. Simultaneous NIR stimulation and extracellular electrical recording are performed based on methods described previously using optical stimulation (Gradinaru et al., *J. Neurosci.*, 27, 14231-14238 (2007)). The electrode consists of a tungsten electrode (1 MΩ; .005 in; parylene insulation) with the tip of the electrode projecting beyond the fiber by 300-500 μm. The electrode is lowered through the NAc in approximately 100 μm increments, and NIR-upconverted optical responses are recorded at each increment. Signals are amplified and band-pass filtered (300Hz low cut-off, 10 kHz high cut-off) before digitizing and recording to disk. At each site, 5 stimulation repetitions are presented and saved.

The examples, which are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way, also describe and detail aspects and embodiments of the invention discussed above. The foregoing examples and detailed description are offered by way of illustration and not by way of limitation. All publications, patent applications, and patents cited in this specification are herein incorporated by reference as if each individual publication, patent application, or patent were specifically and individually indicated to be incorporated by reference. In particular, all publications cited herein are expressly incorporated herein by reference for the purpose of describing and disclosing compositions and methodologies which might be used in connection with the invention. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

EDITORIAL NOTE

Number 2011323231

Please note that the following claim pages are numbered 44 to 46

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of depolarizing or hyperpolarizing the plasma membrane of a neural cell in an individual, wherein the method comprises:

(a) placing a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and

(b) exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (NIR) spectrum, wherein the electromagnetic radiation in the IR or NIR spectrum is upconverted into light in the visible spectrum by the nanoparticles, and wherein a light-responsive opsin is expressed on the plasma membrane of the neural cells and activation of the opsin by the light in the visible spectrum induces the depolarization or hyperpolarization of the plasma membrane,

wherein the light-responsive opsin that induces depolarization comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:6, 7, 8, 9, 10 or 11; and

wherein the light-responsive opsin that induces hyperpolarization comprises an amino acid sequence at least 85% identical to SEQ ID NO:4.

2. A method of depolarizing or hyperpolarizing the plasma membrane of a neural cell in an individual, wherein the method comprises:

(a) administering a polynucleotide encoding a light-responsive opsin to an individual, wherein the light-responsive protein is expressed on the plasma membrane of a neural cell in the individual and the opsin is capable of inducing membrane depolarization or hyperpolarization of the neural cell when illuminated with light;

(b) administering a plurality of lanthanide-doped nanoparticles in proximity to the neural cell; and

(c) exposing the plurality of nanoparticles to electromagnetic radiation in the infrared (IR) or near infrared (NIR) spectrum, wherein the electromagnetic radiation in the IR or NIR spectrum is upconverted into light in the visible spectrum and the activation of the opsin by the light in the visible spectrum induces the depolarization or hyperpolarization of the plasma membrane,

wherein the light-responsive opsin that induces depolarization comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:6, 7, 8, 9, 10 or 11; and

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wherein the light-responsive opsin that induces hyperpolarization comprises an amino acid sequence at least 85% identical to SEQ ID NO:4.

3. The method of claim 1 or claim 2, wherein the light-responsive opsin that induces depolarization comprises an amino acid sequence at least 90% identical to the amino acid sequence set forth in one of SEQ ID NOs:6, 7, 8, 9, 10 or 11.

4. The method of claim 1 or claim 2, wherein the light-responsive opsin that induces hyperpolarization comprises an amino acid sequence at least 90% identical to SEQ ID NO:4.

5. The method of any one of claims 1 to 4, wherein the lanthanide metal is selected from the group consisting of Lanthanum, Cerium, Praseodymium, Neodymium, Promethium, Samarium, Europium, Gadolinium, Terbium, Dysprosium, Holmium, Erbium, Thulium, Ytterbium, and Lutetium.

6. The method of any one of claims 1 to 5, wherein the nanoparticles comprise NaYF4:Yb/X/Gd, wherein X is Er, Tm, or Er/Tm.

7. The method of any one of claims 1 to 6, wherein the nanoparticles are doped nanoparticles.

8. The method of any one of claims 1 to 7, wherein light-emitting diode(s) deliver light at about 700 nm to 1,000 nm.

9. The method of any one of claims 1 to 8, wherein the nanoparticles emit light between 450-550nm.

10. The method of any one of claims 1 to 9, wherein the electromagnetic radiation in the IR or NIR spectrum is:

(a) upconverted into light having a wavelength of about 450 nm to about 550 nm;

(b) upconverted into light having a wavelength corresponding to red, yellow, or amber light; or

(c) upconverted into light having a wavelength corresponding to green or blue light.

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11. The method of any one of claims 1 to 10, wherein the individual is a non-human animal.

12. The method of any one of claims 1 to 10, wherein the individual is a human.

13. The method of claim 11 or claim 12, wherein the skull of the human or non-human animal has been surgically thinned and a source of IR or NIR is placed directly over the thinned-skull region.

14. The method of any one of claims 1 to 13, wherein the neural cell is a neural cell in the central nervous system.

15. The method of any one of claims 1 to 13, wherein the neural cell is a neural cell in the peripheral nervous system.

16. The method of any one of claims 1 to 15 substantially as described herein.

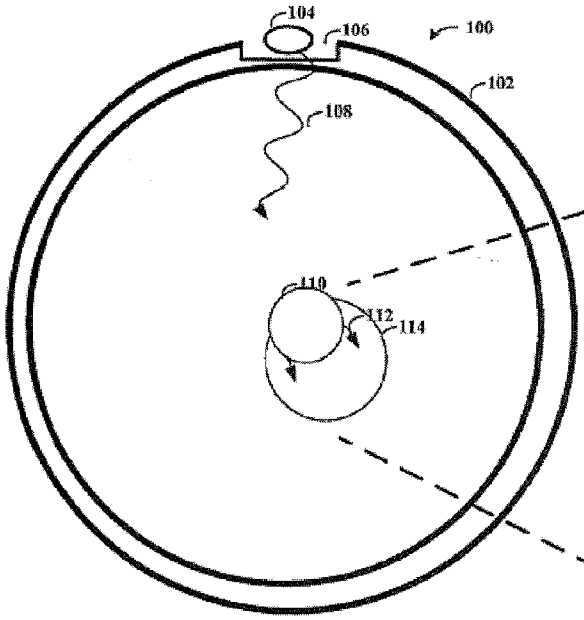
104
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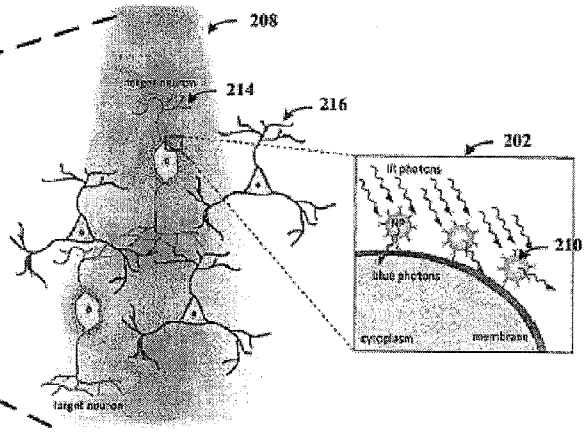
FIG. 1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216
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210
blue photons
cytoplasm
membrane
target neuron

FIG. 2

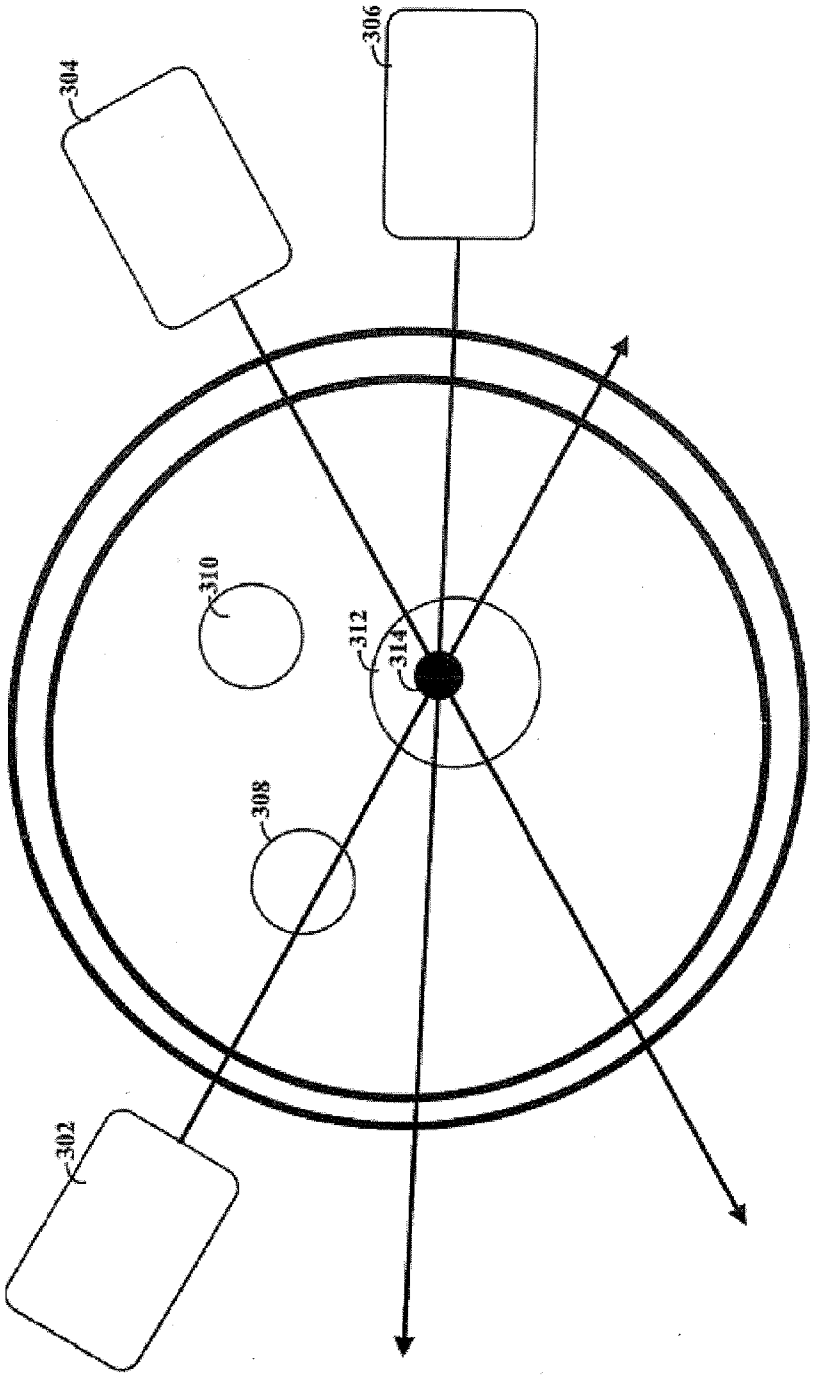

FIG. 3